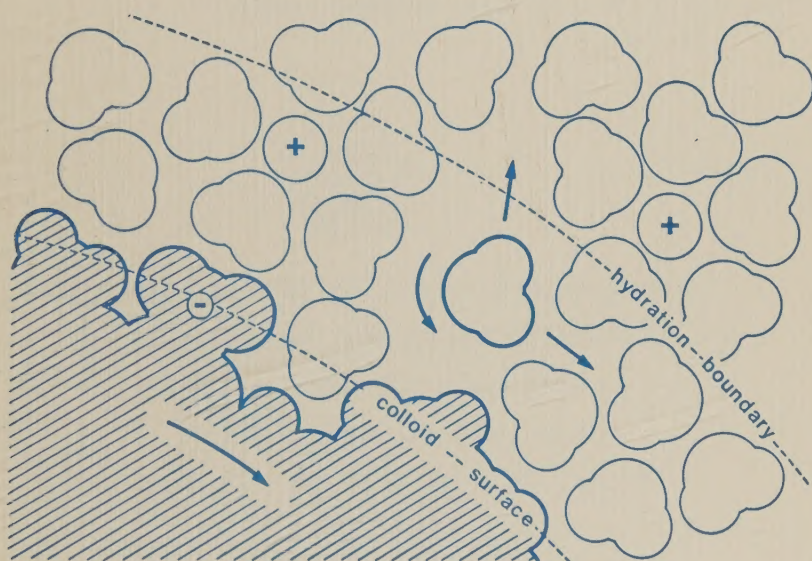


WATER ^{17}O AND ^2H SPIN RELAXATION IN AQUEOUS COLLOIDAL SYSTEMS

LENNART PICULELL



LUND 1985



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Abstract The frequency dependence of the <u>deuteron spin relaxation in supercooled heavy water at 225 MPa</u> was studied, and <u>the results were found to be consistent with isotropic water molecule reorientation</u>. Values of the <u>orientational correlation time and of the librationaly averaged deuterium quadrupole coupling constant</u> were obtained. Water oxygen-17 spin relaxation was used to study water dynamics in solutions of <u>linear polyelectrolytes, polysaccharides, surfactant micelles, globular proteins, and spherical silica particles</u>. The data support a two-state (hydration or bulk water) model for <u>water dynamics in colloidal systems</u>, the hydration state encompassing one or two layers of water molecules. The <u>hydration water molecules reorient rapidly</u>, less than an order of magnitude slower than bulk water molecules, and with a slight preferential orientation with respect to the colloidal surface. <u>Charged surface groups produce the largest perturbations of water reorientation</u>. The slow dynamics contributing to the water oxygen-17 spin relaxation in colloidal solutions were interpreted in terms of a reduced diffusivity of <u>hydration water molecules</u>. In solutions of colloidal silica, the lateral and radial diffusivities were found to be reduced, compared to bulk water, by 1-2 and 2-3 orders of magnitude, respectively. Comparisons of oxygen-17 and deuteron spin relaxation in solutions of proteins, silica particles, and alkylammonium chloride revealed large <u>contributions from prototropic surface groups to the deuteron relaxation</u>.		
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Signature Lennart Piculell

Date March 28, 1985

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- VII. WATER SPIN RELAXATION IN COLLOIDAL SYSTEMS; PART 3.
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B. Halle and L. Piculell
Submitted for publication.

1. INTRODUCTION

*What would you do if I sang out of tune
would you stand up and walk out on me?
Lend me your ears and I'll sing you a song
and I'll try not to sing out of key.*

(John Lennon and Paul McCartney)

Water and aqueous systems are, have been, and probably always will be, subjects of intense research¹. One reason for this might possibly be that it is all too easy to argue why one should study water; I will therefore resist the temptation to give such arguments here. After all, the undisputable status of this ubiquitous chemical has been referred to in too many opening lines in scientific writing already - and how often is not a grand introduction followed by petty discussions of meagre results? I will not take that risk! In all truthfulness, my efforts on the subject of water which are contained in this thesis were not so much inspired by some end goals - whatever these may be - but much more by the excitement of learning, exploring and working together with those who were, and are, my teachers, colleagues, and friends. It was just my luck that it happened to be water and not, say, hydrazine, that became the subject of my research. In any case, I think modesty befits the scientist working on water; he may spend most of his energy keeping his head above it.

The emphasis in my work is on experimental studies of water ^{17}O and ^2H spin relaxation in various aqueous systems, and the aim of these studies was to extract information mainly on the dynamics of water molecules. Max Planck is quoted of having said: "Experiments are the only means of knowledge at our disposal. The rest is poetry, imagination." Indeed, it takes a lot of poetry and imagina-

tion to convert primary data from spin relaxation experiments to conclusions about molecular dynamics, and, undoubtedly, some poets are more imaginative than others. I have been fortunate enough to work with some very good ones, and if there is anything in this work that deserves to be called imaginative or poetic, it is most probably due to them.

My thesis consists of seven papers, listed on page 1, and this summary of their content. It is my prejudice that thesis summaries are rarely very informative but commonly rather dull; as I had no illusions that mine would be an exception in any positive sense, I at least tried to make it short. Having failed to some extent in this respect as well, I strongly recommend the Reader with a genuine interest in the scientific content of this work to immediately turn to paper I and continue his or her reading there. To all others: I can only hope that the present summary makes it clear why you should (or should not) want to read papers I, II, III, IV, V, VI or VII of my thesis.

Mobilis in mobili

Nemo scit quomodo

2. DYNAMICS IN LIQUID WATER

*Around- 'n-around- 'n-a up- 'n down we go again,
oh, baby make me know you love me so an' then,
let's twist again*

(Kal Mann and Dave Appell)

Our present picture of liquid water has been summarized by Stillinger² as "a random hydrogen-bond network, with frequent strained and broken bonds, that is continually subject to spontaneous restructuring". Associated with this restructuring are the reorientational and translational motions of individual water molecules, and it is with these dynamic processes, and how they may be perturbed in the vicinity of colloidal solutes, that this thesis is concerned.

Let us focus on dynamics that involve water molecules as rigid entities, i.e. let us disregard vibrations and proton transfer reactions. The most rapid dynamic processes we encounter in liquid water are then the librational oscillations of a water molecule around some mean orientation in the quasistatic liquid structure. The processes giving rise to changes in this structure occur on a much longer timescale; in pure water at room temperature the characteristic times for molecular reorientation and translation (a distance of the order of one molecular diameter) are both in the picosecond range.

Solutes will perturb the dynamics of nearby water molecules, and I will refer to those molecules measurably perturbed as hydration water. The solutes of interest here are of colloidal dimensions, with translational and reorientational dynamics orders of magnitude slower than those of bulk water molecules. To describe the dynamics of hydration water in colloidal solutions, we should distinguish at least three different processes which may be in-

fluenced, to varying degrees, by the presence of the colloidal surface: the local reorientation of water molecules; the translation of molecules along the surface, and the translation of water molecules away from the surface. In addition, the dynamics of the colloid itself - if sufficiently rapid - may influence the displacements of water molecules in space. The drawing on the cover of this thesis provides a schematic illustration of these different dynamic processes.

Although even a qualitative understanding of the dynamics of hydration water is nowhere near complete, considerable conceptual progress in this respect has been made in recent years^{3,4}. The most general conclusion that can be drawn at present is that hydration water molecules are highly mobile; much more so than was commonly believed, say, a decade ago. Quantitatively, we know most about the local reorientation of hydration water molecules. Experiments from a large variety of systems have shown that this reorientation is very rapid, typically slower by less than an order of magnitude than the corresponding process in bulk water. Furthermore, it has been demonstrated that a surface induces a slight orientational bias for hydration water molecules; i.e. a water molecule, when reorienting next to a surface, is free to assume different orientations with respect to the surface, but some will be more favoured than others. The most illustrative experimental studies on the reorientation and dynamical ordering of hydration water molecules are perhaps those of Woessner, who, by measuring ^2H quadrupolar line splittings⁵ and relaxation rates⁶ in expandable clays of varying water layer thicknesses, was able to show that the measurable surface induced perturbation of water reorientation extends only one or two molecular diameters into the liquid.

A complete description of the dynamics of hydration water will, even in the simplest case, require a consideration of the many different mechanisms by which a surface will influence water molecules. Perhaps it is meaningful to distinguish between static



and dynamic effects: The static effects have their origin in the molecular details of the interface and in differences in bulk properties between the aqueous and the non-aqueous media, which will result in interactions and structure of hydration water molecules differing from those in bulk water. Packing constraints are included in these effects, as well as electrostatic effects at different levels of description. However, even if such differences in structure and interactions between hydration water and bulk water are disregarded, we must recognize that a (more or less) rigid surface creates an environment that is dynamically anisotropic for hydration water. Inasmuch as the translation and reorientation of water molecules are results of collective processes involving many molecules, such processes must necessarily be perturbed in the presence of (rigid) structures that are large compared to a water molecule - irrespective of the detailed nature of such structures. Perhaps it is reasonable to expect some progress in the understanding of the relative importance of these different effects in the near future, as a result of continuing experimental as well as theoretical efforts on the subject of the dynamics of water in colloidal systems.

3. SPIN RELAXATION OF WATER NUCLEI

*What goes up must come down
Spinnin' wheel got to go 'round*

(David Clayton-Thomas)

To study the dynamics of water molecules I have employed the method of nuclear spin relaxation measurements, and the purpose of this section is to briefly review this powerful method, with special emphasis on its application in my own research. The subjects touched upon include basic principles and concepts, methodological considerations, and interpretational models.

3.1 Basic Principles and General Considerations.

An atomic nucleus with a non-zero spin angular momentum and an associated magnetic moment will interact with a magnetic field; furthermore, the spin becomes spatially quantized with respect to the field. An assembly of such nuclei will, when subjected to a static magnetic field, give rise to a net macroscopic equilibrium magnetization aligned with the field. Electromagnetic radiation at the resonance frequency generates transitions between the spin energy levels and a phase coherence of the precessing spins, with a resulting change in the macroscopic magnetization. This is the basis for the well-known NMR phenomenon⁷.

In pulsed NMR⁸, short pulses at the resonance frequency are used to turn the magnetization a well-defined angle away from its equilibrium direction. When perturbed by such a pulse, the spin system will regain thermal equilibrium through fluctuations, often corresponding to molecular motions, in electromagnetic couplings between the spins and their molecular surroundings. Experimentally,

this may be observed as a return of the magnetization to its equilibrium state, and this is, basically, how spin relaxation measurements are performed. When the timescale of (some of) the fluctuations causing the relaxation is of the order of the inverse resonance frequency, or longer, the transverse (orthogonal to the field) component of the magnetization decays faster than the longitudinal (parallel to the field) component. In all cases considered here, the relaxation of either component is an exponential process, characterized by a single, longitudinal or transverse, relaxation rate. (While these are the most commonly measured relaxation rates, others can be obtained by special techniques). The relaxation rates then depend on the strength of the coupling that fluctuates in time, on the timescale(s) of these fluctuations, and on the resonance frequency (which is proportional to the magnetic field) of the experiment.

Once the couplings giving rise to spin relaxation are identified and quantified, the measured relaxation rates yield information on molecular dynamics, and this is one of the main reasons for performing spin relaxation experiments. When the relaxation rates depend on the resonance frequency within the experimentally available range, one may obtain the characteristic time (the so-called correlation time) of the underlying dynamics without knowing the coupling strength.

The virtues of the spin relaxation technique are numerous. Its versatility is the subject of textbooks, not of this short summary. Selectivity is another great advantage; most systems of interest present a choice of nuclei suitable for study, and if there are problems due to overlapping signals from different chemical species, these problems may often be circumvented through isotopic substitution. The method is gentle; very small energies are involved, and due to the weak coupling between the spin and the molecular degrees of freedom, the latter are virtually unperturbed by the measurements. Finally, and perhaps most importantly,

the basic theory of spin relaxation is well developed and rests on solid ground.

3.2 The Water Nuclei.

Water provides three stable isotopes, ^1H , ^2H and ^{17}O , that are amenable to the NMR technique. Due to its high natural abundance and excellent sensitivity, the ^1H nucleus is the most studied of the three, while ^{17}O has been the least popular choice, primarily due to a low natural abundance and to relatively high costs of ^{17}O enriched water. However, today's powerful high field spectrometers require only a modest enrichment from a sensitivity point of view, and in practice all three water nuclei are excellent as far as spectrometer time and sample costs are concerned. Consequently, other considerations should dictate the choice of nucleus, or nuclei, in a particular application.

For the studies reported in this thesis, our choice fell upon the ^{17}O and ^2H nuclei. In the absence of scalar relaxation contributions (cf. below), both these nuclei relax entirely through the coupling of their nuclear electric quadrupole moments with electric field gradients on the nuclei. Of importance here is the fact that in water - even in the presence of monovalent ionic solutes^{4,9} - these field gradients are, to an excellent approximation, of intramolecular origin. To a high degree of accuracy then, the quadrupolar coupling in water fluctuates in time exclusively as a result of the reorientation of individual water molecules. The ^1H nucleus, on the other hand, relaxes through inter- and intramolecular couplings between magnetic dipoles. Molecular translation as well as rotation will then contribute to the water ^1H relaxation. In colloidal solutions, the information on water dynamics from ^1H relaxation is further obscured by the well-documented effects of cross-relaxation between water protons and protons of the colloidal solute.

The ^1H nucleus shares another disadvantage with the otherwise excellent ^2H nucleus: When (colloidal) solutes in the studied aqueous system contain prototropic groups, these may, through rapid proton (deuteron) exchange, contribute to the observed water ^1H (^2H) signal. It is often difficult to know *a priori* whether such contributions will be present or not, let alone to estimate their magnitude. The ^{17}O nucleus then remains as the only generally safe source of information on water dynamics from spin relaxation studies in colloidal solutions.

There are however, potentially complicating factors even with ^{17}O relaxation. One is due to the high spin quantum number ($I = \frac{5}{2}$) of the nucleus: When slow motions contribute significantly to the relaxation, the longitudinal and transverse components of the ^{17}O magnetization will not only decay at different rates, but also, in principle, decay as sums of exponential functions, rather than as single exponentials. In practice, however, the relaxation is often found to be singly exponential even when the transverse rate exceeds the longitudinal rate. In this case, approximate analytical expressions for the apparent rates may be used¹⁰. The errors in such a treatment may be evaluated, and are often found to be negligible - as in all cases studied here.

Another complication is the scalar contribution to the transverse ^{17}O relaxation rate in water. This effect is also referred to as the proton-exchange broadening of the ^{17}O linewidth, as it is due to a modulation of the scalar (spin-spin) coupling between ^{17}O and ^1H (^2H) through water proton (deuteron) exchange. The scalar contribution is substantial in pure water around neutral pH, where the proton exchange is slow^{11,12}. However, the purely quadrupolar relaxation is easily obtained from measurements well outside the neutral pH range, or by the use of decoupling techniques. Moreover, as is illustrated in paper II in this thesis (cf. also below), the scalar contribution is actually a blessing in disguise, as it may in fact be utilized to extract information on water dynamics.

3.3 Orientational Correlation Functions.

The terms '(time) correlation function' and 'correlation time' are often encountered in the literature on spin relaxation. Taking an orientational time correlation function as an example, this function can be understood as an answer to the question: Given that a reorienting object has a specified orientation in space at time zero, what is the probability that it has the same orientation a time t later? In certain special cases, such as isotropic Brownian motion, the correlation function decays exponentially with time, and the characteristic decay time is then defined as the correlation time. When the form of the correlation function is complicated or if it is unknown, the correlation time may be defined as the integral of the correlation function.

A spin relaxation rate is often expressed as a function of one or more correlation times, as it depends on, among other things, the correlation function for the fluctuating coupling that gives rise to the relaxation. For ^2H and ^{17}O in water, which relax through intramolecular couplings, the correlation functions describe the reorientation of single water molecules. In pure water, the loss of orientational correlation of the molecules is a result of two statistically independent processes, *viz.* the damped librations and the overall tumbling of the molecules. The orientational correlation function then displays an initial very rapid decrease due to damped librations, followed by a slower, nearly exponential decay due to overall reorientation.

In colloidal solutions, the above two processes are not sufficient to completely average the orientation of a water molecule. The reason for this is that solvent molecules spend part of their time at the colloid surface, which, as we saw earlier, induces a slight preferential orientation in the hydration water.

This remaining orientational correlation can be lost in three different ways: The colloid will tumble and average its special orientation, water molecules may move laterally along the colloid surface and average their positions on the colloid, and finally, a water molecule will eventually lose all correlation with the colloid by diffusing away from it. The final decay of the orientational correlation function for water molecules in colloidal solutions is then determined by (the most rapid of) these three dynamic processes, which henceforth will be referred to as slow processes.

3.4 The Two-Step Formalism

The colloidal systems investigated here are isotropic on the NMR timescale; more precisely, this means that the timescale of at least one of the slow, isotropic processes is short compared to the inverse of the interaction that remains to be averaged by slow processes after partial averaging by rapid, local motions. Both the fast and the slow motions will then contribute to the observed relaxation, and as the two classes of motions occur on different timescales, one can treat the relaxation within the so-called two-step formalism⁴. In this formalism, the total correlation function is expressed as a sum of two parts, referring to the statistically independent dynamic "steps".

As we saw earlier, the timescale separation also applies to the librational and reorientational motions of water molecules in the pure liquid, and thus the two-step formalism may also be applied in this case, to account for the partial librational averaging of the quadrupole coupling in water. In the papers contained in this thesis, we have made use of the two-step formalism to treat both of the above cases.

3.5 The Two-State Discrete Exchange Model.

As we have seen, water molecules in colloidal systems sample different environments, or states, characterized by differing dynamics and differing orientational probability distributions. However, it is often the case (as in all the studies in this thesis) that the water NMR signal from a colloidal system is due to all (observable) water molecules in that system; i.e. water molecules in the various states do not give rise to different observable signals. Therefore, the measured relaxation rates also characterize all observed water molecules. It is then common practice to recognize only two states: One 'free' state, characterized by bulk water properties, and one 'bound' state, which contains all water molecules measurably perturbed by the colloidal solute.

Under certain conditions it may be meaningful to describe the dynamics in each state by an intrinsic correlation function, and one can then define intrinsic relaxation rates for the two states. If the exchange between the states is rapid compared to the most rapid intrinsic relaxation rate, the observed relaxation rates may be expressed as population weighted sums of the intrinsic rates¹³. This interpretational model, which is referred to as the discrete exchange model (DEM), is commonly applied to water relaxation in colloidal systems. Although the two-state model and the DEM are often used together, one should recognize that they are based on distinct assumptions about the system.

As only average properties are observed, the NMR relaxation rates will not allow a separation of the extent and the range of the perturbation of water molecules; in terms of the two-state DEM, one cannot separately deduce the fraction of bound water molecules and their intrinsic relaxation rates.

4. SUPERCOOLED HEAVY WATER

*Slow down, you move too fast
You got to make the morning last*

(Paul Simon)

No currently available experimental method yields a full description of the reorientation of water molecules even in the pure liquid. The most detailed information at hand derives instead from molecular dynamics computer simulations^{14,15}. However, at the present time, such results are semi-quantitative, at best, as the intermolecular pair potentials that are used are quantitatively inaccurate and insufficient to account for many-body effects on water reorientation. Another weakness in current practice, if not in principle, is that computer simulations only follow a fairly small number of water molecules over rather limited time spans.

In principle, orientational correlation functions for water molecules may be obtained from ^2H and ^{17}O spin relaxation measurements over the range of resonance frequencies where the relaxation rates are frequency dependent, i.e. where the resonance frequency is of the order of the inverse of the orientational correlation time. Unfortunately, such a study of water in the stable liquid range would require spectrometers equipped with $10^3 - 10^4$ T magnets! (The magnetic fields of currently available spectrometers extend to ca. 15 T). However, Lang and Lüdemann have recently shown^{16,17} that at sufficiently low temperatures (around -80°C), the water reorientation in supercooled liquid water is slowed down to rates which are within the range of resonance frequencies that may be reached today. In pure liquid water, such low temperatures can only be achieved at pressures around 2000 atmospheres, where the homogeneous nucleation temperature, T_H (where water inevitably

freezes) goes through a minimum¹⁸. Detailed water spin relaxation studies are thus limited to a rather exotic corner of the water phase diagram. However, the properties of supercooled water, especially at temperatures close to T_H , are of considerable intrinsic interest¹⁹. It is, furthermore, noteworthy that other methods aimed at extracting dynamic information (including computer simulations²⁰) encounter great difficulties when applied to water near T_H .

The above considerations provide the background to the study presented in *paper I* of this thesis. There we report an extension of the previous study by Lang and Lüdemann¹⁷ on ^2H spin relaxation in supercooled heavy water at high pressures. Whereas the latter work was limited to longitudinal relaxation at a single resonance frequency (15.35 MHz), paper I presents longitudinal and transverse relaxation rates at two additional frequencies (39.14 MHz and 55.54 MHz) in $^2\text{H}_2\text{O}$ under a pressure of 225 MPa and at temperatures down to -85°C . The combined data of paper I and ref. 17 will then serve as a better testing ground for models of dynamics in supercooled water than previously available results. We found, in paper I, that the simplest conceivable model of water reorientation, i.e. one of isotropic rotation involving a single orientational correlation time, was consistent with all experimental data. With no further assumptions, we could calculate the librationaly averaged deuterium quadrupole coupling constant, as well as the orientational correlation time, in the temperature range where the relaxation is frequency dependent. All measured relaxation rates in the investigated temperature range (188-286 K) could be satisfactorily reproduced with a librationaly averaged coupling constant of 201 kHz and with an empirical equation for the temperature dependence of the correlation time.

5. COLLOIDAL SYSTEMS: WHICH AND WHY

*I don't need to hug or hold you tight
I just wanna dance with you all night*

(John Lennon and Paul McCartney)

In general terms, the aims of the last six papers of this thesis were (i) to gain further insight into the dynamics of water molecules in colloidal solutions, (ii) to study specific systems of interest and to see whether they conform to a general picture of such dynamics and (iii) to quantify contributions from prototropic groups to the measured ^2H relaxation rates in various colloidal systems - the last point being warranted by a wealth of $^1\text{H}/^2\text{H}$ investigations where such contributions have not been given adequate attention. The studied systems contain colloidal solutes of three different kinds: linear polyelectrolytes (papers II and III), micellar aggregates of ionic surfactants (paper IV), and spherical polyelectrolytes (papers V, VI and VII). In this section I will discuss these papers individually, while the next section is devoted to general conclusions that may be drawn from all the studies.

5.1 Linear Polyelectrolytes

In *paper II*, we chose to investigate, using ^{17}O relaxation, the water dynamics in solutions of two linear polycarboxylic acids, *viz.* poly(acrylic acid) (PAA) and poly(methacrylic acids)(PMA). PMA and PAA are among the best studied synthetic polyelectrolytes available, and as they can be converted, in solution, from highly charged to uncharged polymers by simple acid/base titration, they were regarded as model systems, well suited for our purposes. We measured the dependence of the ^{17}O relaxation rates on temperature, polymer concentration, and degree of dissociation (α) of the carboxylate

groups, and we were able to interpret all data in terms of a two-state DEM, using the two-step formalism to describe the relaxation in the 'bound' state. For PAA we invariably found that the longitudinal and transverse relaxation rates were equal, indicating that all dynamic processes contributing to the water relaxation were rapid compared to the resonance frequency (34.56 MHz). Furthermore, linear concentration dependences of the ^{17}O relaxation at $\alpha = 0$ and $\alpha = 1$ demonstrated that these dynamics are independent of polymer concentration in the investigated range (up to *ca.* 0.6 mol monomer/kg H_2O). Moreover, the ^{17}O relaxation rate was found to increase monotonically with increasing α .

For PMA at $\alpha \gtrsim 0.5$, the results were qualitatively similar to those of PAA, whereas, at lower degrees of dissociation, PMA gave rise to a frequency dependence as well as a non-linear concentration dependence in the ^{17}O relaxation, revealing effects of interpolymer interactions on the observed water dynamics. These results were related to a well-documented conformational transition of PMA occurring in the low α range. The dependences on α of the ^{17}O relaxation rates in PMA/PAA solutions were qualitatively different from the corresponding dependences of the ^2H relaxation rates, which have been reported previously^{21,22}. This was expected, since -COOD contributions have been found²² to dominate the ^2H relaxation enhancement, relative to pure water, in solutions of PMA/PAA at $\alpha < 1$.

Comparison of the PMA and PAA results with results from solutions of the respective monomers showed that in all cases, except for PAA at $\alpha = 0$, the polyacids affect the water dynamics more than the monomers do. Finally, we studied the suppression of the ^{17}O scalar relaxation rate, relative to its pure water value, in solutions of PMA/PAA. This effect is due to a catalysis of the water proton exchange by carboxylic acid groups, and from our results we could deduce upper limits to the lifetimes of water molecules 'bound' to these groups. (Very recently, Lankhorst and Leyte²³ have published a more extensive study of the water -

carboxylic acid proton exchange kinetics in solutions of PMA/PAA. Their results, based on measurements of the scalar contribution to the transverse ^1H relaxation in water enriched in ^{17}O , are in qualitative agreement with ours, although there are quantitative differences, amounting to a factor of 2, in the rate constants obtained.)

In *paper III*, we turned our attention to biological polyelectrolytes, studying solutions of hyaluronate (Hy) and chondroitin (Ch), which belong to the class of charged polysaccharides commonly referred to as glycosaminoglycans. Paper III contains studies of counterion ($^{23}\text{Na}^+$, $^{25}\text{Mg}^{2+}$) as well as water ^{17}O relaxation, the main objective being to analyse how specific features of the investigated polysaccharides would be reflected in dynamics of molecules in the aqueous component (as opposed to the polyelectrolyte itself) of the systems. The interpretation of counterion relaxation in colloidal systems is closely analogous to the interpretation of water relaxation outlined in section 3, an important difference being that, in the case of counterion relaxation, one also has to consider the effects of a non-uniform distribution of the ions in polyelectrolyte solutions.

The results of paper III show that previously observed alkali-induced changes in rigidity of the Hy molecule are accompanied by changes in counterion and water relaxation. Qualitatively similar, but quantitatively smaller effects were found in solutions of Ch, an epimer of Hy. These results were interpreted in terms of deprotonation of polysaccharide hydroxy groups at alkaline pH. Just as in the PMA/PAA case, all Hy/Ch data were consistent with a two-state model for water (and ion) relaxation, and observed changes in counterion relaxation in various titration experiments could be explained in terms of variations in the fraction of 'bound' ions as a result of changing Coulomb interactions.

5.2 Dodecylammonium Chloride

Paper IV is a short letter concerned with the interpretation of ^2H relaxation rates from aqueous alkylammonium halide solutions. We measured the ^{17}O and ^2H relaxation rates in a micellar solution of dodecylammonium chloride, before and after addition of a small amount of hydrochloric acid. In the pure salt solution, both ^2H relaxation rates were found to contain significant contributions from ammonium deuterons, exchanging rapidly with the water deuterons. These contributions disappeared on addition of acid, and the intrinsic relaxation rates in the alkylammonium headgroups could thus be evaluated.

5.3 Spherical Polyelectrolytes

In the last three papers of the thesis, we try to attack, in a systematic way, the problem of the slow dynamics (on a time-scale of 10^{-8} seconds or longer) contributing to water spin relaxation in solutions of globular proteins and other more or less spherical colloids. Prior to discussing these papers, let us first establish why such slow dynamics present a problem; indeed, slow water correlation times were in a sense easier to understand, say, a decade ago, when the term 'bound water', as applied to colloidal systems, was not commonly put within quotation marks. A water molecule was then often believed to be more or less rigidly attached to a colloidal particle for some (long) time, the lifetime of bound water. During this time, it would reorient with the colloid, and the effective correlation time of a bound water molecule would be determined by (the most rapid of) the dynamic processes, colloid reorientation and exchange of water molecules between the bound and the free states. This picture leads directly to the DEM for water spin relaxation.

However, as we now know that water molecules next to surfaces are very mobile indeed, the above simple picture becomes clearly unrealistic: A water molecule at a surface, reorienting nearly isotropically on a timescale of 10^{-11} seconds, cannot have a lifetime, in the above sense, of 10^{-8} seconds. All we can say with certainty, then, is that the final loss of orientational correlation of a water molecule residing at the surface of a colloid can occur in three different ways (cf. above): Through reorientation of the colloid, through averaging of the position of the water molecule on the surface of the colloid (e.g. by surface diffusion), or through diffusion of the water molecule away from the colloid to some point where the probability of returning (with some orientational correlation retained) is zero.

Since the respective timescales of these processes should have differing dependences on the size of the colloidal solute, we decided to measure water spin relaxation in a system which allowed the particle size to be varied independently, and over a reasonably wide range. The spherical, rigid, nonporous and well-characterized particles of alkali-stabilized colloidal silica meet this requirement, and in *paper V* we present an extensive study of water ^{17}O and ^2H spin relaxation in silica sols of varying particle diameter (from *ca.* 10 nm to 80 nm). From the frequency dependence of the relaxation rates the contributions from fast and slow dynamics could be separated, and while the fast dynamics were found to be independent of the size of the colloids (confirming the size independence of the particle surface structure), the contribution from slow dynamics increased monotonically with particle diameter. A slow correlation time of the order of 10^{-8} - 10^{-7} seconds could be inferred from the weak frequency dependence (over the accessible range) of the relaxation. All dynamics contributing to the water relaxation were found to be independent of particle concentration. From comparison of the ^2H and ^{17}O relaxation we were able to conclude that the ^2H relaxation

contained large contributions from silanol groups, exchanging deuterons rapidly with the water - so rapidly, in fact, that the slow correlation time of the ^2H relaxation was found to be entirely determined by water dynamics.

Further experimental information regarding the slow dynamics contributing to water spin relaxation in colloidal systems is provided in *paper VI*, where we compare the ^{17}O and ^2H relaxation in solutions of globular proteins. This investigation was prompted by a series of detailed studies of water ^1H and ^2H relaxation in protein solutions by Koenig and coworkers²⁴. These reports constitute a particularly rich source of dynamic information as the workers were able to map out the entire low-frequency dispersion of the longitudinal relaxation in their systems. Consequently, the slow correlation time could be accurately determined. An interpretation in terms of water dynamics was, however, not without problems; thus the DEM in its most primitive form (with a single, slow correlation time describing the dynamics of bound water) was found to lead to inconsistencies regarding the lifetime of bound water. One objective of paper VI was to reinterpret the data of Koenig et al. using the more recently developed two-step formalism for the relaxation of 'bound' water, and, indeed, with a more realistic picture of the reorientational freedom of hydration water the apparent inconsistencies were found to disappear.

Another finding of Koenig et al. was that the slow correlation time of water $^1\text{H}/^2\text{H}$ relaxation increases monotonically with protein size. Thus, in the case of hemocyanin - which was the largest protein investigated - a slow correlation time of *ca.* 10^{-6}s was obtained. However, the problem of possible contributions from exchangeable protein protons/deuterons to the measured rates was not resolved unequivocally, and it was thus never demonstrated to what extent the measured correlation times give information on solvent water dynamics. To settle this question,

we compared, in paper VI, the ^{17}O and ^2H relaxation in solutions of lysozyme and hemocyanin under varying conditions, and we could indeed establish additional contributions to the ^2H rates, as compared to the ^{17}O rates, in both these systems. In the case of lysozyme, the differing pH dependence of the ^{17}O and ^2H relaxation rates demonstrated rather directly that the extra contribution comes from prototropic protein sidechains. From a qualitatively different temperature dependence of the ^{17}O and ^2H transverse relaxation rates in a solution of hemocyanin, we could establish that, also in this system, the ^2H relaxation was largely determined by a contribution absent in the ^{17}O relaxation. More importantly, from the frequency dependence of the ^{17}O relaxation in hemocyanin we could conclude that the slow ^{17}O correlation time was at least an order of magnitude smaller than the slow ^2H correlation time documented by Koenig et al.

With the rather extensive experimental information from papers V and VI at hand, we could, in *paper VII*, venture a general interpretation of the contribution from slow dynamics to the water ^{17}O relaxation in colloidal systems. To this end, we made use of a recently developed model for spin relaxation in locally ordered fluids^{4,25} and applied this model to the experimental data of paper V. The interpretational model features the two-step formalism for spin relaxation, coupled with a continuous diffusion model (CDM) for the translational motion of water molecules in colloidal solutions. In the CDM, as in the DEM, two states (B and F) characterized by different dynamics and orientational probability distributions for water molecules are recognized; however, intrinsic relaxation rates for the two states cannot, in general, be defined. To obtain the contribution from slow processes to the relaxation, one instead solves the diffusion equation for water molecules in a cell containing one colloidal particle and an amount of water corresponding to the concentration of the solution. Moreover, the diffusiv-

ity tensor is allowed to vary; whereas, in the F state, the diffusivity is the same as in bulk water, the B state diffusivity may be different, and its radial and lateral components (relative to the colloidal surface) may differ. Apart from being more general than the DEM, the CDM has the following advantages: (i) Effects of reencounters of water molecules with the surface after their initial stay there are treated explicitly, and (ii) only well-defined dynamic parameters are involved; thus, the diffusivity tensor for hydration water may be obtained from other types of measurements and from computer simulations.

We show in paper VII that the model applied can explain all the ^{17}O relaxation data from our colloidal silica model system (paper V), provided that the lateral and radial diffusivities of water molecules within ≈ 1 nm of the colloid surface are reduced, compared to the bulk water value, by 1-2 and 2-3 orders of magnitude, respectively. More rapid water diffusion in the B state invariably led to qualitative or quantitative disagreement between experiment and theory. We also included the effects of the reorientation of the colloidal particle on the water dynamics, and found that such effects had a negligible influence on the water ^{17}O relaxation for particle radii in excess of ≈ 10 nm.

In the limit of very slow radial diffusion in the B state, the CDM reduces to the DEM, whereby simple expressions for the slow contributions to the water relaxation obtain. In view of the success of the DEM in accounting for experimental data, we also attempted an explanation of the long residence time of hydration water in terms of an effective potential barrier rather than in terms of a reduced radial diffusivity. However, such a model was found to be inconsistent with the observed activation energy for the relaxation rate.

As protein molecules in many ways represent a more complex model system than silica particles, we could not make a detailed analysis, similar to that of the silica sol data, of the available water ^{17}O relaxation data from protein solutions. However, the general features of the slow water dynamics were found to be quite similar in protein and silica solutions.

6. COLLOIDAL SYSTEMS: GENERAL CONCLUSIONS

*Well, she was just seventeen,
and you know what I mean,
and the way she looked was way beyond compare.
So how could I dance with another, woo,
when I saw her standing there.*

(John Lennon and Paul McCartney)

6.1 ^2H versus ^{17}O .

In papers IV, V and VI a comparison of (the pH dependence of) ^{17}O and ^2H relaxation in colloidal systems revealed contributions from prototropic groups to the observed ^2H relaxation rates. When such contributions were present, they were found to dominate the contributions from slow dynamics to the ^2H relaxation. From these studies, two conclusions may be drawn:

- (i) If the colloidal solute contains acidic functions, one should be very cautious to draw conclusions on water dynamics based on ^2H data alone.
- (ii) The non-water contributions to the ^2H relaxation may be separated (through comparison with ^{17}O relaxation) and are thus a source of additional dynamic information.

6.2 Rapid Hydration Water Dynamics

By applying the two-step formalism, we were able to estimate the effective correlation time for the rapid, local reorientation of hydration water in solutions of polycarboxylic acids (paper II), dodecylammonium chloride (paper IV) and colloidal silica (paper V). In all cases, this correlation time was larger than that of bulk water by no more than a factor of 10, in agreement with previous

results⁶. For carboxylic acid groups (papers II and III) and silanol groups (paper V) we found that the deprotonated, charged forms have larger retarding effects on the rapid water dynamics than the uncharged do.

6.3 Slow Hydration Water Dynamics

In all investigated cases, the DEM was found to give a good description of water ^{17}O relaxation. This was also the case for silica sols, where the obtained dynamic parameters of a more general model, the CDM, were found to reduce the model to the DEM limit (paper VII). In the silica system, a lateral diffusion of hydration water molecules, slower by one or two orders of magnitude than that of bulk water, had to be invoked to account for the experimental observations. The residence time of hydration water was found to be in the range of 10^{-8} - 10^{-7} seconds both in protein and in colloidal silica solutions (papers V-VII).

General conclusions regarding the slow dynamics in linear polyelectrolyte solutions are more difficult to draw (cf. also below), as the contributions from slow dynamics to the ^{17}O relaxation were seen to vary with polymer conformation and/or concentration (papers II and III). However, upper limits, of the order of 10^{-8} s, to the lifetime of water molecules bound to carboxylic acid groups could be obtained from measurements of scalar relaxation contributions to the ^{17}O relaxation (paper II).

In a micellar solution of dodecylammonium chloride at 43 °C, (paper IV) only rapid water dynamics were found to contribute significantly to the water ^{17}O and ^2H relaxation rates, thus precluding quantitative conclusions on slow water dynamics in this system as well.

6.4 The Two-State Model

In all investigated systems, the two-state model for water in colloidal systems was found to give a good description of the data. However, one cannot be certain, *a priori*, that the slow and the fast dynamics report on the same bound water molecules; a comparatively small fraction of motionally very restricted water molecules could be responsible for a large part or all of the observed slow dynamics, while contributing negligibly to the rapid dynamics (cf. the contribution from prototropic groups to the ^2H relaxation, which often dominates the transverse relaxation, while being similar in magnitude to the hydration water contribution in the case of longitudinal relaxation). In concentrated polymer systems, such as those of papers II and III, this possibility cannot be ruled out. However, in protein solutions, the ratio of the fast and slow contributions to the ^{17}O relaxation was found to be remarkably constant for a large number of different proteins (paper VII), which indicates that both contributions report on 'normal' hydration water. For silica sols, we found that the slow and the fast contributions varied in a similar way with pH, which again supports the two-state model. Indeed, if rapid lateral positional averaging of water molecules occurs during their residence time at a colloidal surface, which our results indicate, then a two-state model seems all the more appropriate.

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*Mm, I'm gonna try with a little help from my friends
Oh, I get high with a little help from my friends
Yes, I get by with a little help from my friends,
with a little help from my friends*

(John Lennon and Paul McCartney)

If it is difficult to write a thesis summary that is short and yet inclusive, similar ambitions regarding acknowledgements are impossible. A mere list of all you people who have contributed to making my apprenticeship years of scientific endeavour pleasant and rewarding might end up looking like an excerpt from a telephone directory - and each one of you would deserve some personal, spiritual comment. I give up, cheat, and address all of you with a deeply felt "Thank you for everything". However, I still wish to mention a few persons who have been especially special to me in special ways; thank you

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IV

On Water ^{17}O and ^2H Spin Relaxation in Alkylammonium
Chloride Solutions

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Running title: ^{17}O and ^2H NMR in Surfactant Solutions

ABSTRACT

Water ^{17}O and ^2H longitudinal and transverse spin relaxation rates in aqueous (100% D_2O) micellar solutions of dodecylammonium chloride ($\text{C}_{12}\text{ND}_3\text{Cl}$) are reported. Both ^2H relaxation rates contain significant contributions from ammonium headgroup deuterons, exchanging rapidly with bulk water. On addition of acid, these contributions disappear. The intrinsic ^2H rates in the headgroups may thus be evaluated. A comparison of the ^{17}O and ^2H rates in the absence of headgroup contributions shows that the intrinsic relaxation rates of hydration water report on local water dynamics.

INTRODUCTION

Recent years have seen a rapid increase in the number of reports on water spin relaxation rates in surfactant systems¹⁻⁵. In such studies, the ^2H and ^{17}O nuclei are generally preferred, since these nuclei relax by intramolecular quadrupolar interactions, yielding information directly related to single water molecule motion. However, care has to be exercised in the interpretation of ^2H data from systems containing prototropic residues, since these may exchange deuterons rapidly with bulk water and thus give significant (or even dominant) contributions to the measured relaxation rates⁶⁻¹¹. Such contributions can be evaluated in a straightforward way by comparison of the pH dependence of the ^{17}O and ^2H rates^{10,11}. By this method we will, in the present letter, demonstrate - in conflict with the conclusion of a recent study⁵ - that the water ^2H relaxation rates in aqueous solutions of alkylammonium chloride contain significant contributions from deuterons residing in the surfactant headgroups. (Exchange effects on the ^2H quadrupolar line splittings in the lamellar phase of octylammonium chloride have been demonstrated previously¹².) Our study will exemplify that the combined ^{17}O and ^2H data, in the presence and in the absence of deuteron exchange effects, may provide rather detailed information on water and surfactant head-group dynamics in aqueous solutions of prototropic surfactants.

Experimental Section

Dodecylammonium chloride ($C_{12}ND_3Cl$) was prepared as described by Néry et al.¹³. The sample at pH 3.32 (cf. Table I) was obtained by dissolving the pure salt in D_2O , enriched to 0.5% in ^{17}O , and will be referred to as pure $C_{12}ND_3Cl$ solution. The sample at pH 0.94 was obtained by adding a small amount of DCl to the pure sample, and will be referred to as acidified $C_{12}ND_3Cl$ solution. Relaxation rates were measured at 34.6 MHz (^{17}O) and 39.1 MHz (2H). Longitudinal rates (R_1) were measured by inversion recovery. Transverse rates (R_2) were obtained from linewidths (corrected for 4 s^{-1} inhomogeneity broadening) of absorption spectra (^{17}O) or measured by the Carr-Purcell-Meiboom-Gill pulse sequence (2H). The measurements were performed at $43 \pm 0.5^\circ C$ to permit direct comparison with previously published $R_1(^2H)$ data from $C_{12}ND_3Cl/D_2O$ mixtures⁵.

Results and Discussion

Table I lists ^{17}O and 2H longitudinal and transverse relaxation rates at $43^\circ C$ in pure and acidified dodecylammonium chloride micellar¹³ solutions. It is seen that while the ^{17}O rates are unaffected by the addition of acid, both 2H rates change substantially. Furthermore, in pure $C_{12}ND_3Cl$ solution, $R_2(^2H)$ is much larger than $R_1(^2H)$, whereas, in the acidified sample, the two 2H rates are equal - as are the ^{17}O rates in both samples. These results demonstrate that, in pure $C_{12}ND_3Cl$ solution, there are significant contributions to both 2H rates from surfactant head-

group deuterons, exchanging rapidly with the water. The deuteron exchange is catalyzed by a very small fraction (10^{-3} - 10^{-4} as estimated from the measured pH) of deprotonated headgroups. In the acidified sample, however, effectively all headgroups are in the acid form, and the deuteron exchange is slow compared to the intrinsic relaxation rates (cf. below) of the ammonium deuterons.

In order to assess to what extent quantitative conclusions on hydration water dynamics from ^2H data may be affected by the ammonium contributions, we make the usual analysis of our data in terms of a two-state discrete exchange model in the rapid exchange limit^{14,15}. Thus, if the measured relaxation rates are entirely due to water molecules exchanging rapidly between the "bound" (B) and "free" (F) states, the conventionally defined excess relaxation rates may be written as

$$R_{i\text{ex}} \equiv R_i - R_F = P_B (R_{iB} - R_F) \quad (i = 1, 2) \quad (1)$$

where R_{iB} and R_F are the intrinsic relaxation rates in the respective states, and P_B is the fraction of water molecules in the B state.

To facilitate comparison of data from different water nuclei, we will normalize the excess rates with respect to the bulk water (or "free" water) rate:

$$\frac{R_{i\text{ex}}}{R_F} = P_B \left(\frac{R_{iB}}{R_F} - 1 \right) \quad (2)$$

Finally, the fraction of bound water molecules may be expressed as

$$P_B = n \frac{w_S}{w_W} \frac{M_W}{M_S} \quad (3)$$

where n is the number of bound water molecules per surfactant molecule, w_S/w_W is the surfactant-to-water weight ratio, and M_W and M_S are the molar masses.

Using equations (1) - (3), we may obtain the quantities $n\left(\frac{R_{iB}}{R_F} - 1\right)$ from the data of Table I. The results are listed in Table II. It is seen that the normalized ^2H rates from the acidified $\text{C}_{12}\text{ND}_3\text{Cl}$ sample are in good agreement with the normalized ^{17}O rates from both samples, and from the mean value of these data we obtain $n\left(\frac{R_B}{R_F} - 1\right) = 5.7$ for water in a $\text{C}_{12}\text{ND}_3\text{Cl}$ solution. This value should be compared with the ^2H data from pure $\text{C}_{12}\text{ND}_3\text{Cl}$ solution, given in Table II and in ref. 5. The latter work reports measurements of $R_1(^2\text{H})$ as a function of the surfactant-to-water weight ratio in $\text{C}_{12}\text{ND}_3\text{Cl}/\text{D}_2\text{O}$ mixtures at 43°C , and from the linear dependence obtained we deduce $n\left(\frac{R_{1B}}{R_F} - 1\right) = 11 \pm 3$, in quantitative agreement with the corresponding value in Table II. Thus, we conclude that the water and ammonium contributions to the ^2H longitudinal excess relaxation rates in solutions of pure $\text{C}_{12}\text{ND}_3\text{Cl}$ are of similar magnitude.

In ref. 5, results of isotopic dilution experiments were taken to demonstrate the absence of significant contributions to $R_1(^2\text{H})$ from ammonium deuterons. However, as long as the fraction of deuterons residing in ammonium groups is reasonably independent of the isotopic composition of the solvent, it is expected (cf. eq. (3)) that the ratio $R_{i\text{ex}}/R_F$ should be insensitive to isotopic dilution, in the presence as well as in the absence of headgroup contributions to the measured rates.

The ^{17}O data and the ^2H data from the acidified sample of Table II show that the water contributions to the normalized transverse and longitudinal excess relaxation rates of ^{17}O and ^2H in $\text{C}_{12}\text{ND}_3\text{Cl}$ solution are equal. This need not always be the case in solutions of colloids or surfactant aggregates. When slow processes such as micelle reorientation or water diffusion within or out of the B state contribute significantly to the water relaxation, the normalized ^{17}O and ^2H rates will differ, since normalized contributions from slow processes will be roughly a factor of 3 larger for ^{17}O than for ^2H (cf. ref. 15). (Depending on the timescale of the slow processes, and on the resonance frequency of the experiment, R_1 and R_2 may also differ). The results of Table II thus indicate that only rapid, local reorientation of water molecules contributes significantly to the intrinsic relaxation rate in the B state. The ratio R_B/R_F then equals τ_B/τ_F , where τ_B and τ_F are the correlation times describing reorientation of water molecules in the respective states.

The situation is qualitatively different for the intrinsic relaxation rates of ^2H in ammonium headgroups, as is evident from the ^2H data from pure $\text{C}_{12}\text{ND}_3\text{Cl}$ solution in Table II. While it has been demonstrated⁵ that slow processes contribute negligibly to $R_{1\text{ex}}(^2\text{H})$ in alkylammonium chloride solutions, Table II shows that $R_{2\text{ex}}(^2\text{H})$ - at least in micellar solutions - is dominated by these processes. The information on head-group dynamics contained in the ^2H relaxation rates in solutions of pure alkylammonium chloride is readily extracted once the water contribution to the rates is obtained from measurements at sufficiently low pH. An expression completely analogous to eq. (1) should hold for the ammonium

contribution, and since there is no ambiguity as to the fraction of deuterons residing in the headgroups, the intrinsic ammonium rates may be evaluated without further assumptions. In our pure sample we thus obtain $R_1(\text{ND}_3^+) \approx 7 \text{ s}^{-1}$ and $R_2(\text{ND}_3^+) \approx 75 \text{ s}^{-1}$. The difference $R_2 - R_1$, reflects the dynamics of deuteron exchange, micelle reorientation and/or surfactant diffusion - whichever process is the most rapid. Thus, a careful study of the pH dependence of the ^2H relaxation rates in alkyl-ammonium chloride solutions will yield information on deuteron exchange dynamics and surfactant dynamics in addition to the information obtained on water.

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Table I. Water ^{17}O and ^2H Relaxation Rates in $\text{C}_{12}\text{ND}_3\text{Cl}/\text{D}_2\text{O}$
(surfactant-to-water weight ratio = 0.295) at 43°C .

Sample	pH	$R_1(^{17}\text{O})$ (s^{-1})	$R_2(^{17}\text{O})$ (s^{-1})	$R_1(^2\text{H})$ (s^{-1})	$R_2(^2\text{H})$ (s^{-1})
D_2O	2.1	115^{+3}	115^{+2}	$1.61^{+0.07}$	$1.47^{+0.08}$
$\text{C}_{12}\text{ND}_3\text{Cl}$	3.32	134^{+3}	134^{+2}	$2.05^{+0.09}$	$4.58^{+0.20}$
$\text{C}_{12}\text{ND}_3\text{Cl}$	0.94	129^{+3}	133^{+2}	$1.76^{+0.08}$	$1.78^{+0.10}$

Table II. Normalized (see text) ^{17}O and ^2H Excess Relaxation Rates
in $\text{C}_{12}\text{ND}_3\text{Cl}$ Solutions at 43°C .

pH	$n \left(\frac{R_{iB}}{R_{iF}} - 1 \right)$			
	$R_1(^{17}\text{O})$	$R_2(^{17}\text{O})$	$R_1(^2\text{H})$	$R_2(^2\text{H})$
3.32	6 ± 1	6 ± 1	10 ± 3	73 ± 15
0.94	5 ± 1	6 ± 1	4 ± 2	7 ± 3

V

WATER SPIN RELAXATION IN COLLOIDAL SYSTEMS

Part 1: ^{17}O and ^2H Relaxation in Dispersions of Colloidal
Silica.

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Running title: ^{17}O and ^2H NMR in Silica Sols

ABSTRACT

Extensive water ^{17}O and ^2H spin relaxation data from alkali-stabilized aqueous dispersions of spherical colloidal silica particles are presented. Longitudinal (R_1) and transverse (R_2) relaxation rates of both nuclei have been measured in samples of varying particle diameter (ca. 10 to 80 nm) as functions of resonance frequency, temperature, alkali content and particle concentration. Qualitative and quantitative differences in the results from the two nuclei demonstrate that surface silanol groups, exchanging deuterons very rapidly with bulk water, contribute to $R_1(^2\text{H})$ and dominate $R_2(^2\text{H})$. At the highest measured resonance frequencies, the enhancement of R_1 relative to bulk water is entirely due to rapid, local dynamics of water molecules (and, for ^2H , silanol groups), whereas R_2 contains additional contributions from slow diffusive processes. The fast and slow dynamics are independent of particle concentration. The R_1 relaxation enhancement depends only on the ratio of silica surface area to water volume, independently of particle size, indicating that the structure of the particle surface is size invariant. In contrast, the slow dynamics contributing to R_2 has a systematic dependence on particle size.

1. INTRODUCTION

In the last decades, water spin relaxation has emerged as perhaps the most powerful method for extracting information on the dynamics of water molecules in colloidal solutions. The literature on various applications is vast and rapidly growing. The aim of the present series of papers is not only to add to the body of available data by presenting new and extensive ^{17}O and ^2H measurements in particular systems, but, equally importantly, to attempt to draw more general conclusions from such measurements. We will then make special reference to two questions which have been the source of much discussion regarding the interpretation of, in particular, water ^2H relaxation rates in colloidal systems.

One of these questions is: To what extent do relaxation data from the hydrogen isotopes in aqueous colloidal solutions contain contributions from species other than water; i.e. do prototropic groups on the colloid surface, through rapid proton/deuteron exchange with bulk water, provide an efficient relaxation pathway for water protons/deuterons? There is, of course no general answer to this question, but we will make the point that the problem of proton exchange has been taken too lightly in many past ^1H or ^2H NMR studies. In the majority of cases it has not been attacked in the most straightforward way, which is a systematic and extensive comparison of ^{17}O and ^2H relaxation rates in the same samples. Such a comparison should yield an unambiguous answer, since these two nuclei relax through intramolecular mechanisms, in contrast with the ^1H isotope. In bulk water, ^{17}O and ^2H relaxation measurements give virtually the same information on water reorientation¹, and the relaxation behaviour of the two nuclei in the presence of a perturbing surface has been treated in a general theory² which has extensive experimental support. A separation of contributions from water and from prototropic groups

to the ^2H relaxation may also yield interesting information on the chemistry and the dynamics of the colloid surface, which is illustrated by the present study. Thus a major purpose of this article and of the second part of this series³ (henceforth referred to as Part 2), is to present experimental data necessary to make a thorough comparison of the information provided by ^2H and ^{17}O relaxation in two different aqueous colloidal systems, viz. colloidal silica (the present article) and globular proteins (Part 2).

The second question we will address is: How can one account for the slow processes (on a timescale of 10^{-8} seconds or longer) contributing to water ^{17}O and ^2H relaxation in solutions of globular proteins^{4,5}, and, as we shall see, in colloidal silica? In particular, are processes on such long timescales compatible with the picture of rapid reorientation of water next to a surface which has emerged from recent spin relaxation experiments in heterogeneous systems^{4,18}? We will find that the answer to this our second question is connected with the answer to the first one, as the contributions from (and the molecular origin of) the slow processes, in general, differ for ^{17}O and ^2H . However, as ^{17}O relaxation data confirm slow contributions to the correlation function characterizing water reorientation in the studied colloidal systems, we devote the third part of this series⁷ (henceforth referred to as Part 3) to a detailed discussion of the molecular origin of these observations.

Previously, both questions stated above have been discussed⁸ with reference to spin relaxation data from protein solutions. Clearly, it is desirable to compare such data with data from a simpler model system. In particular, as previous work^{4,5} indicates

that the size of the colloid has consequences for the water relaxation, a model system is desirable where particle size can be varied independently, i.e. without varying the nature of the surface, a criterion that is not met by (aggregates of) globular proteins. The surface should be homogeneous, nonporous, and well-characterized. Furthermore, the model colloid of preference should be spherical, rigid and monodisperse, to necessitate the least number of parameters to describe the dynamics in the system, and dispersions should be stable at high enough concentrations to yield large effects on the water relaxation. Possibly, the model system that best meets all of the above requirements is colloidal silica, which we chose for the present study. Methods of obtaining alkali-stabilized silica sols of any desired particle size in the range from 5 to 150 nm have been known for more than thirty years, and during this time such sols have been thoroughly characterized⁹. Particles of this type seem to be compact and nonporous⁹, and from viscosity measurements¹⁰ and alkali titration experiments^{11,12} it has been concluded that the microscopic nature of the surface is independent of particle size. The only disadvantage of the system is a varying degree of polydispersity, which however, does not seriously affect our conclusions.

Although various manifestations of water interactions with the amorphous silica surface have been studied extensively, reports of immediate relevance to the present work are few. This is mainly because most previous studies of water-silica interactions and dynamics deal with the neutral, or nearly so, pure silica surface, whereas the

alkali stabilized particles of our study have a high negative surface charge density, and consequently, a high local concentration of positive counterions immediately outside the surface. To our knowledge, the only previous report on water interactions with the charged amorphous silica surface is a viscosity study by Iler and Dalton¹⁰. As to previous water spin relaxation work, interpretation of the large bulk of ^1H data is complicated by the possibility of cross relaxation between water protons and silanol groups. However, very recently Hanus and Gillis¹³ have reported water ^1H and ^2H longitudinal relaxation rates in dispersions of silica powder as functions of silica concentration and sample temperature, in qualitative agreement with the corresponding measurements of the present work. Again, quantitative agreement is not expected, since the silica powder studied by Hanus and Gillis was not charged. This may also mean that contributions from silanol groups to the observed ^2H relaxation rates are negligible (cf. below), although this was not demonstrated.

To the best of our knowledge, the present work is the most detailed study on water dynamics in colloidal silica yet performed, presenting extensive water ^{17}O and ^2H relaxation data on seven sols, of particle size varying from ca. 10 to 80 nm. Thus, it is our hope that the study should be of interest to the reader with a particular interest in the colloidal silica system, as well as to the reader mainly concerned with the more general problems outlined above. In the following, we present longitudinal and transverse ^{17}O and ^2H relaxation rates as functions of particle size and concentration, alkali content, temperature, and resonance frequency. The discussion in the present article

will concern the conclusions that can be drawn from a comparison of the data from the two nuclei and, also, estimates of the local water mobility near the silica surface, while an interpretation of the slow processes and their dependence on particle size will be given in Part 3.

2. EXPERIMENTAL

2.1 Materials

Dispersions of alkali-stabilized colloidal silica of specific area varying between 68 and 500 m²g⁻¹ were a generous gift from EKA AB, Surte, Sweden. Six of the sols were made by EKA AB, two of which are available commercially as EKASOL 15 (500 m²g⁻¹) and Bindzil GP (241 m²g⁻¹). The seventh sol is Ludox HS40 (226 m²g⁻¹) made by Dupont. The EKA particles contain the following impurities, given as weight percent: Cl(0.1%), Al(0.04%), Ti(0.02%), Fe(0.01%). The copper content is below the detection limit (<0.006%). The sols were characterized by surface titration, BET N₂ adsorption, and electron microscopy at EKA AB, and by dynamic light scattering at this laboratory. Table 1 gives sol characteristics, as well as the concentrations and measured pH of the solutions used in all experiments except those reported in table 2 and figure 2. The pH values are given for comparison between different sols only, as the absolute values measured in concentrated sols are of little significance¹⁵. To the stock solutions, used without further purification, was added isotopically enriched water to a final isotopic composition of 10% ²H and 0.5% ¹⁷O (of the water), unless otherwise stated.

2.2 Relaxation Measurements

^{17}O and ^2H relaxation rates were measured at resonance frequencies ranging from 4.1 to 55.5 MHz. The following spectrometers were used: A Bruker BKr-322s with a Varian V-71 computer (^{17}O and ^2H at the three lowest fields), a modified Varian XL-100-15 (^{17}O at 13.6 MHz), a home-built spectrometer equipped with a 6T magnet (^{17}O at 34.6 MHz, ^2H at 39.1 MHz), and a Nicolet NIC-360 (^{17}O at 49.0 MHz, ^2H at 55.5 MHz). Longitudinal relaxation rates (R_1) were measured by inversion recovery, and transverse relaxation rates (R_2) were obtained from linewidths (unless otherwise stated) according to $R_2 = \pi\Delta\nu$, where $\Delta\nu$ is the linewidth at half amplitude of the absorption spectrum, corrected for inhomogeneity broadening (ca. 2Hz). In all experiments, reference solutions of slightly acidic water with known, frequency independent relaxation rates were measured, and the error estimates given in tables and figures are based on the reproducibility of these measurements. Excess relaxation rates (cf. eq. 4) were evaluated as $R_{i\text{ex}} = R_i - R_{i\text{ref}}$ ($i=1,2$), where $R_{i\text{ref}}$ is the relaxation rate of a reference solution of the same water ^2H enrichment as the sample. It was assumed that the inhomogeneity contributions to sample and reference linewidths cancel. The temperature was kept constant to $\pm 0.5^\circ\text{C}$ by passage of dry thermostated air or nitrogen through the probe.

2.3 Systematic Errors

Time dependence. It is conceivable, especially for a sol with a large specific area, that the particle size changes with time, due to silica dissolving from small particles and depositing on large ones⁹. We therefore carefully checked for any time dependence of the relaxation

rates, and no such time dependence was observed in the four months during which the measurements were made.

Scalar relaxation. Modulation of the scalar coupling between ^{17}O and ^1H (^2H) through proton (deuteron) exchange will, in general, contribute to the transverse relaxation of water nuclei¹⁵. The respective scalar relaxation rates depend on the exchange rates and on the isotopic composition of the water. In water of the isotopic compositions used here, and at quoted pH values, contributions from scalar relaxation to the ^{17}O and ^2H rates are negligible^{16,17}.

Paramagnetic relaxation. Traces of paramagnetic impurities in silica may give non-negligible contributions to the water relaxation rates. Proton magnetic resonance spectra provide a sensitive test for such contributions¹⁸, since these should be 42.4 times larger for ^1H than for ^2H . For the $226\text{m}^2\text{g}^{-1}$ and $133\text{m}^2\text{g}^{-1}$ sols of table 1, ^1H linewidths recorded at 100 MHz and 30°C were 9.3 Hz and 12.1 Hz respectively, which should be compared with ^2H linewidths of 14.0 Hz and 11.9 Hz at 39.1 MHz and 27°C (figure 4). Thus, paramagnetic contributions to the ^2H and ^{17}O relaxation rates are insignificant.

Nonexponential relaxation. The relaxation of ^{17}O ($I = \frac{5}{2}$) is strictly exponential only under extreme narrowing conditions, i.e. when $R_2 = R_1$ and the relaxation is independent of resonance frequency. However, as long as R_2/R_1 is of order one, the relaxation will be effectively exponential and approximate analytical expressions for the measured rates can be used¹⁹. The errors in such a treatment can be evaluated¹⁹, and in the present case they correspond to less than 2.5% in $R_2(^{17}\text{O})$ for the worst case, while the treatment of $R_1(^{17}\text{O})$ is virtually exact.

Effects of isotopic composition. Since the water used in this study is a mixture of the isotopes ^1H , ^2H , ^{16}O , and ^{17}O , the ^{17}O and ^2H relaxation rates will strictly monitor the reorientation of different isotopic species. Thus, in the present study the ^2H rates will mainly report on $^2\text{H}^1\text{H}^{16}\text{O}$, and the ^{17}O rates on $^1\text{H}_2^{17}\text{O}$. For our purposes, the difference in dynamics of these species²⁰ is completely negligible.

3. THEORETICAL

The theoretical model outlined here has been applied previously to water spin relaxation in heterogeneous systems^{4,6}, and is discussed further in Parts 2 and 3. We interpret the relaxation rates in terms of the usual discrete exchange model in the fast exchange limit, according to which the measured relaxation rates, R_i , may be written as²¹

$$R_i = P_F R_F + P_B R_{iB} \quad (i = 1, 2) \quad (1)$$

where P_F and P_B are the fractions of free and bound water, respectively, the free state being defined as the solution less the dispersed particles. In the case of ^2H we will allow for the possibility of a third state (subscript P), comprising the deuterons on the silanol groups, which may exchange rapidly with bulk water. The effects of the P state can (under certain circumstances; cf. section 5.1) be accounted for by an additional term $P_P R_{iP}$ in eq. (1). In the B and P states, motions on two timescales will, in general, contribute to the relaxation: The largest part of the quadrupolar interaction will be averaged out by

rapid, local motions (effective correlation times τ_{Bf} and τ_{Pf}) that are more or less anisotropic, leaving a remainder to slow isotropic processes (effective correlation times τ_{Bs} and τ_{Ps}). A theoretical formalism for the spin relaxation in such a case has been developed, with special emphasis on water nuclei², and on nuclei residing on the surface of spherical colloids^{22,23}. The formalism is quite general and can be combined with specific models for the underlying interactions and dynamics. A discussion of such models is given in Part 3; for the present purposes, we only need the final expressions for the relaxation rates. If, as is often the case, the measurements are performed at a resonance (angular) frequency ω_0 such that $\tau_{Bf} \ll \omega_0^{-1} \ll \tau_{Bs}$, then the relaxation rates in the B state are given by²

$$R_{1B} = \frac{3\pi^2}{10} \frac{2I+3}{I^2(2I-1)} \chi^2 \left(1 + \frac{\eta^2}{3} - A_B^2\right) \tau_{Bf} \quad (2a)$$

$$R_{2B} = \frac{3\pi^2}{10} \frac{2I+3}{I^2(2I-1)} \chi^2 \left[\left(1 + \frac{\eta^2}{3} - A_B^2\right) \tau_{Bf} + \frac{3}{10} A_B^2 \tau_{Bs} \right] \quad (2b)$$

In these expressions, I is the nuclear spin quantum number, χ is the quadrupole coupling constant and η is the asymmetry parameter of the field gradient tensor. A_B^2 , the square of the residual anisotropy², is a measure of the orientational correlation remaining to be averaged out by slow motions after the initial rapid decay of the correlation function due to local motions. If $\tau_{Pf} \ll \omega_0^{-1} \ll \tau_{Ps}$, the expressions for the P state relaxation rates are completely analogous to eq. 2. In the F state, extreme narrowing obtains, and

$$R_F = R_{1F} = R_{2F} = \frac{3\pi^2}{10} \frac{2I+3}{I^2(2I-1)} \chi^2 \left(1 + \frac{\eta^2}{3}\right) \tau_F \quad (3)$$

Finally, we define the excess relaxation rates

$$R_{iex} \equiv R_i - R_F \quad (i = 1,2) \quad (4)$$

4. RESULTS

4.1 Frequency Dependence

Table 2 lists relaxation rates measured at several resonance frequencies for a dispersion with $A_{sp} = 226 \text{ m}^2\text{g}^{-1}$ (cf. Table 1). The differences between R_1 and R_2 demonstrate contributions from slow motions to the relaxation, and the frequency dependence of the relaxation rates within the range of frequencies studied shows that the slow motions occur on a timescale longer than 10^{-8}s . Note that contributions from slow motions to the longitudinal relaxation are not negligible at the lower frequencies. This observation is of some relevance to a recent study of water ^2H relaxation in dispersions of silica powder¹³, where the authors made the tacit assumption that at 33°C and at a resonance frequency of 9.2 MHz, only the local, rapid dynamics contribute to the longitudinal relaxation. However, the main conclusion from table 2 is that the frequency dependence is weak in the measured range and negligible at the highest frequencies. The latter statement is confirmed by the results of figure 3 (cf. below). Thus, when discussing the results

given below, which were all obtained at high resonance frequencies, we may use the simple expressions given in eq. 2 for the relaxation rates.

4.2 Temperature Dependence

The temperature dependence of the relaxation rates was measured to assess the validity of the fast exchange assumption underlying eq. 1. If the nuclei exchange rapidly between the states, an Arrhenius plot of $\ln R_{2ex}$ versus $1/T$ should yield a curve of positive slope. Data for three sols displayed in fig. 1 shows that this is indeed the case. For comparison, we also give the rates in pure water at the same isotopic composition.

4.3 Concentration Dependence

For a given sol, the fraction of bound water, P_B , is proportional to the weight ratio W_{SiO_2}/W_{H_2O} . According to eqs. (1) and (4), the excess relaxation rates should also be proportional to W_{SiO_2}/W_{H_2O} , provided that the relaxation rate in the bound state is not concentration dependent. We have performed dilution experiments on one large particle ($68 \text{ m}^2\text{g}^{-1}$) and one small particle ($226 \text{ m}^2\text{g}^{-1}$) sol, the results of which are given in figure 2. To facilitate comparison, the data of figure 2 have been normalized with respect to the relaxation rates in bulk water (as given in Fig. 1) of the same isotopic composition and at the same temperature. As is evident from the plots, the intrinsic relaxation rates of both ^2H and ^{17}O at the silica surface are indeed independent of particle concentration, which, according to eq. (2),

means that the slow as well as the fast motions are independent of interparticle distance within the studied range.

4.4 Particle Size Dependence

Figures 3 and 4 give normalized ^{17}O and ^2H excess relaxation rates, respectively, for the seven different sols of table 1, obtained at two different temperatures. The R_2 data at 27°C of figure 3 were measured at two frequencies, confirming the frequency independence (table 2) of the relaxation rates at these frequencies. It is easily verified (cf. also eq. (9) below) that if the number of bound water molecules per unit surface area is independent of the particle size, then P_B is proportional to $A_{\text{sp}} W_{\text{SiO}_2} / W_{\text{H}_2\text{O}}$. To facilitate comparison between the different sols, the excess relaxation rates have been divided by this factor. The linear concentration dependences demonstrated in fig. 2 make such a procedure valid.

A number of interesting features are evident in the collective data of figs. 3 and 4. First, we note that the ^{17}O and ^2H data show qualitative similarities, although there are quantitative differences in the normalized relaxation rates; small, but significant, in $R_{1\text{ex}}$, and very large in $R_{2\text{ex}}$.

A second observation, which may appear trivial at first, is that the P_B -normalized longitudinal excess relaxation rates of figures 3 and 4 are independent of particle size. However, this observation gives support to the following assumptions made above: (i) That the surface structure of the silica particles is independent of their size, and (ii) That the excess longitudinal relaxation rates, at these fre-

quencies, measure the local, rapid water reorientation, which is independent of particle size. In contrast, the normalized transverse rates, which contain contributions also from slow dynamics, increase markedly with particle size. Our interpretation of the latter observation is given a detailed discussion in Part 3.

A third point which deserves attention is the temperature dependence of the relaxation rates. Figures 3 and 4 show that the excess longitudinal relaxation rates scale with the rates in bulk water, whereas the excess transverse rates of the larger sols vary less rapidly with temperature (cf. also fig. 1).

4.5 Alkali Titration

So far, all the ^{17}O and ^2H data presented show qualitative similarities. However, fig. 5 shows that the relaxation rates of the two nuclei in a silica sol respond differently to addition of alkali. Both ^{17}O relaxation rates increase on addition of alkali, which, from similar experiments on proteins⁴ and linear polyacids²⁴, is the expected effect of an increase in the number of charged groups on the surface. In contrast, the transverse relaxation of ^2H decreases monotonically, the decrease being larger than expected from the dilution of the sample due to addition of titrant. The scatter in $R_1(^2\text{H})$ is too large to reveal any tendency.

Two conclusions may be drawn immediately from figure 5. The first is that the effect of changing the pH over the studied range ($9.59 \leq \text{measured pH} \leq 10.26$) is small on all relaxation rates. This means that the small variation in pH of the sols (cf. table 1) should not in-

validate the comparisons made in figures 3 and 4. The other conclusion, to which we will return in the next section, is that the qualitative difference in the response of the two nuclei to addition of alkali is a clear indication that the major contribution to the ^2H transverse relaxation does not originate from bound water molecules.

5. DISCUSSION

5.1 Comparison of ^2H and ^{17}O Relaxation

A likely explanation of the different trends of the ^2H and ^{17}O relaxation rates in figure 5 is that deuterons in surface silanol groups are responsible for most of the ^2H excess transverse relaxation. The reduction of $R_2(^2\text{H})$ on addition of alkali should then correspond to deprotonation of silanol groups*. To get an upper limit for the magnitude of this effect, let us assume that one silanol group will be deprotonated for each added OH^- ion. Knowing the total weight and the specific area of the silica particles in the sample, we calculate from the amount of added NaOH that the entire titration in figure 5 would then correspond to removing 0.4 silanol protons/deuterons per square nanometer of silica surface. The total number of titratable silanol

*For reasons that are not clear to us, the charge bearing species on the alkali stabilized silica surface are often referred to as "adsorbed hydroxyl ions" in the silica literature⁹. We consider it natural to identify the charged groups as deprotonated silanol groups, and have done so throughout. Indeed, the decrease in $R_2(^2\text{H})$ on addition of alkali favours this interpretation; adsorption of hydroxyl ions should, if anything, give rise to an increase in all measured relaxation rates.

groups per square nanometer is not known with certainty, but a reasonable estimate appears to be in the range $5-8^9$. From this we should subtract the number of groups that are charged at the beginning of the titration, which, according to the Na content of the sol (table 1) should amount to 1 per nm^2 . From these considerations we can estimate the maximum reduction in the number of silanol deuterons due to the titration in figure 5 to be 6 - 10%. The observed reduction of $R_{2\text{ex}}(^2\text{H})$ is 6.8%, and the estimated trivial dilution effect is 2.6%.

Apart from the qualitative differences evident from fig. 5, the quantitative differences between the normalized excess relaxation rates of ^2H and ^{17}O (figs. 2-5) are evidence of additional contributions to the ^2H rates. The correlation times τ_{Bf} and τ_{Bs} should be the same for both nuclei, and as it is generally found that $A_{\text{B}}^2 \ll 1$ for water², equations (2)-(4) tell us that $R_{1\text{B}}/R_{\text{F}}$ should be the same for both nuclei. The normalized longitudinal excess relaxation rates, however, differ significantly (figs. 2-5). This discrepancy is seen more clearly in the difference between the transverse and longitudinal relaxation rates. From equations (1) and (2) we see that for ^{17}O , where only the B and F states exist, the difference between the transverse longitudinal relaxation rates is given by

$$\Delta R \equiv R_2 - R_1 = \frac{9\pi^2}{100} \frac{2I+3}{I^2(2I-1)} P_{\text{B}} X^2 A_{\text{B}}^2 \tau_{\text{Bs}} \quad (5)$$

and dividing by equation (3), we see that for ^{17}O , the ratio $\Delta R/R_F$ depends on the correlation times τ_{BS} and τ_F , the residual anisotropy A_B and the asymmetry parameter η . The latter two parameters depend on the nucleus, and using the values² $\eta(^2\text{H}) = 0.11$, $\eta(^{17}\text{O}) = 0.93$ and $[A_B(^{17}\text{O})/A_B(^2\text{H})]^2 = 4$, we estimate the water contribution to $\Delta R(^2\text{H})/R_F(^2\text{H})$ to $0.3\Delta R(^{17}\text{O})/R_F(^{17}\text{O})$. From the ^2H and ^{17}O data in figures 2-5 it then follows that the expected water contribution to $\Delta R(^2\text{H})$ amounts to less than 1% of the measured effect.

Ascribing the entire difference $\Delta R(^2\text{H})$ to the P state, the ratio of the normalized relaxation rate differences of the two nuclei becomes

$$\frac{\Delta R(^2\text{H})R_F(^{17}\text{O})}{R_F(^2\text{H})\Delta R(^{17}\text{O})} = \left[1 + \frac{[\eta(^{17}\text{O})]^2}{3} \right] \cdot \frac{P_P [\chi_P(^2\text{H})]^2 [A_P(^2\text{H})]^2}{P_B [\chi_F(^2\text{H})]^2 [A_B(^{17}\text{O})]^2} \cdot \frac{\tau_{PS}}{\tau_{BS}} \quad (6)$$

where we have neglected the asymmetry parameter $\eta_P(^2\text{H})$. Table 3 lists this ratio as calculated from the data of figures 2-4. It is seen that the ratio is independent of particle size and temperature, although for each nucleus, there is considerable variation in $\Delta R/R_F$ with particle size as well as with temperature (cf. figs. 3 and 4). Whereas it is expected that the fractions of nuclei in the B and P states, the coupling constants, and the residual anisotropies should be virtually independent of particle size and temperature, this is not so for the ratio of the correlation times, unless these describe the same dynamic process(es) for both nuclei and $\tau_{PS} = \tau_{BS}$. One instance when $\tau_{PS} = \tau_{BS}$ is when the slow correlation times for both hydration water and exchanging protons in surface prototropic groups are completely determined by particle reorientation

correlation time τ_{rot}), as is the case in solutions of small and medium-sized proteins (cf. Part 2). However, for colloids of the size studied here, it is found (Parts 2 and 3) that τ_{rot} is too long to influence $\Delta R(^{17}\text{O})$ significantly; rather it is determined by translational diffusion of water molecules relative to the colloid surface. From the results of Table 3, we must then conclude that, in alkali-stabilized silica sols, the slow correlation time of the ^2H relaxation also describes bound water dynamics. This conclusion is compatible with the finding that silanol groups give the dominant contribution to $\Delta R(^2\text{H})$ only if the lifetime of a deuterium on a silanol group is very short compared to τ_{BS} .* From the data of Table 1 we concluded that $\tau_{\text{BS}} > 10^{-8}$ s at room temperature. To get an estimate of the lifetime of a proton on a silanol group, we assume that the lifetime of a deprotonated silanol group is of the order of the lifetime of an hydroxide ion in water, which is ca. 10^{-12} s at room temperature¹⁷. Per deprotonated silanol group, there should be less than 10 that are protonated (cf. above), and the lifetime of a silanol proton would then be of the order of 10^{-11} s. Assuming that proton exchange between silanol groups always occurs via water (which is reasonable judging from the surface density of silanol groups⁹), we may indeed expect that the silanol-water proton exchange is very rapid compared to τ_{BS} .

*A consequence of this interpretation is that the B and P states can be regarded as effectively one state as far as $\Delta R(^2\text{H})$ is concerned. In eq. (6), P_p should then be replaced by $P_p + P_B$, and A_p should be replaced by an average residual anisotropy for all ^2H nuclei in the B and P states². However, given the uncertainties in P_B and P_p , we will not pursue the analysis of $\Delta R(^2\text{H})$ any further.

5.2 Water Mobility near the Silica Surface

From equations (2a), (3) and (4) we get, for ^{17}O ,

$$\frac{R_{1\text{ex}}}{R_F} = P_B \left(\frac{R_{1B}}{R_F} - 1 \right) = P_B \left(\frac{\tau_{Bf}}{\tau_F} - 1 \right) \quad (8)$$

where the last equality follows since $A_B^2 \ll 1$. The fraction of bound water molecules can be written as

$$P_B = n \cdot \frac{M_{\text{H}_2\text{O}}}{N_A} \cdot \frac{W_{\text{SiO}_2}}{W_{\text{H}_2\text{O}}} \cdot A_{\text{sp}} \cdot 10^{18} \quad (9)$$

where n is the number of bound water molecules per square nanometer, $M_{\text{H}_2\text{O}}$ is the molar mass of water (gmol^{-1}) N_A is Avogadro's number and A_{sp} is the specific area of the silica particles (m^2g^{-1}). For the evaluation of n , we note that there is convincing experimental support^{6,18} for the notion of a short-ranged dynamical perturbation of water molecules at an interface, extending one or two molecular layers into the liquid. We thus estimate $10 \lesssim n \lesssim 20$, and using the mean value $\langle (R_{1\text{ex}}/R_F)(W_{\text{H}_2\text{O}}/W_{\text{SiO}_2})/A_{\text{sp}} \rangle = 2.64 \times 10^{-3} \text{m}^{-2}\text{g}$ from all data in figure 3, we calculate $9.8 \gtrsim \tau_{Bf}/\tau_F \gtrsim 5.4$, independently of particle size and temperature. For the same ratio in a sodium hectorite clay Woessner¹⁸ obtained the value 5.35 from ^2H water relaxation, assuming a monolayer of bound water.

5.3 The Residual Anisotropy

From the linear concentration dependences of figure 2, we obtain $R(^{17}\text{O})_{\text{H}_2\text{O}}/W_{\text{SiO}_2} = 101 \text{ s}^{-1}$ for the $226 \text{ m}^2\text{g}^{-1}$ sol. With this value and with $\chi(^{17}\text{O}) = 6.67 \text{ MHz}$, equations (5) and (9) yield $n[A_{\text{B}}(^{17}\text{O})]^2\tau_{\text{BS}} = 1.16 \text{ ns}$. Fits of the full, frequency dependent expressions for the longitudinal relaxation rates (see e.g. Parts 2 and 3 of this series) to the data of table 2 for the same sol, yield values for τ_{BS} in the range 30 - 120 ns. As before, we estimate $10 \leq n \leq 20$, and obtain $0.02 \leq |A_{\text{B}}(^{17}\text{O})| \leq 0.06$, a typical^{4,7} range of values for the water ^{17}O residual anisotropy in charged colloidal systems.

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Table 1. Data on the investigated silica sols.

A_{sp}^a (m^2g^{-1})	Particle diameter (nm)				poly- dispersity ^f	$\frac{W_{Na_2O}}{W_{SiO_2}}$ $\times 10^3$	$\frac{n_{Na}}{nm^2}^g$	$\frac{W_{SiO_2}}{W_{H_2O}}$	pH ^h
	$D_{A_{sp}}^b$	D_{BET}^c	D_{EM}^d	D_{DLS}^e					
500	5.5	5.5		21.4	0.72	26.0	1.0	0.159	10.2
360	7.6			24.0	0.34	12.7	0.7	0.276	9.6
241	11.3		13	28.5	0.32	10.3	0.8	0.381	9.5
226	12.1		18	24.2	0.30	10.8	0.9	0.595	9.8
133	20.5		35	40.7	0.14	7.0	1.0	0.600	9.5
78	35.0	46	53	61.2	0.06	5.5	1.4	0.362	9.5
68	40.1	56	80	82.5	0.10	3.5	1.0	0.600	9.6

^aFrom NaOH titration according to Sears¹¹.

^bFrom A_{sp} with $\rho_{SiO_2} = 2.20 \text{ kgdm}^{-3}$

^cFrom BET N_2 adsorption

^dFrom electron microscopy

^eFrom dynamic light scattering

^fFrom DLS; defined as the normalized variance of the particle diffusion coefficients.

^gNumber of Na per square nanometer, calculated from W_{Na_2O}/W_{SiO_2} and A_{sp} .

^hGiven as the pH meter readings from measurements directly in the sols.

Table 2. Frequency dependence of ^{17}O and ^2H relaxation rates at 25°C in an aqueous (25% ^2H , 2.5% ^{17}O) dispersion of colloidal silica ($W_{\text{SiO}_2}/W_{\text{H}_2\text{O}} = 0.248$; $A_{\text{sp}} = 226 \text{ m}^2\text{g}^{-1}$).

^{17}O			^2H		
ν_0 (MHz)	R_1 (s^{-1})	R_2 (s^{-1})	ν_0 (MHz)	R_1 (s^{-1})	R_2^{a} (s^{-1})
4.067	194 ± 10		4.605	4.12 ± 0.21	
8.134	192 ± 10		9.210	3.26 ± 0.16	
12.201	187 ± 9		13.816	2.94 ± 0.15	
13.565		212 ± 4			
34.566	173 ± 9	201 ± 4	39.141	2.45 ± 0.12	21.7 ± 2.2
49.046	$171 \pm 9^{\text{b}}$	$208 \pm 4^{\text{b}}$	55.537	$2.58 \pm 0.13^{\text{b}}$	$21.4 \pm 2.2^{\text{b}}$

^aFrom Carr-Purcell-Meiboom-Gill pulse sequences.

^bMeasurements were made at 28°C , and the data have been normalized to 25°C in the following way:

Data obtained at 28°C have been divided by the factor 0.90 corresponding to the mean of $R_{1\text{ref}}(^{17}\text{O})$ at 49.046 MHz, 28°C , divided by the mean of the same quantities obtained at the lower frequencies at 25°C .

Table 3. Ratio of normalized relaxation rate differences for ^{17}O and ^2H in silica sols. Data from figures 2-4.

A_{sp} (m^2g^{-1})	$[\Delta R(^2\text{H})R_F(^{17}\text{O})] / [\Delta R(^{17}\text{O})R_F(^2\text{H})]$		
	4 $^{\circ}\text{C}$	23 $^{\circ}\text{C}$	27 $^{\circ}\text{C}$
500	45		49
360	45		38
241	49		38
226	46	49	46
133	48		50
78	46		42
68	46	50	47

FIGURE CAPTIONS

- Fig. 1. Temperature dependence of the ^{17}O (34.6 MHz) and ^2H (39.1 MHz) excess transverse relaxation rates in aqueous (10% ^2H , 0.5% ^{17}O) dispersions of colloidal silica of varying specific area ($A_{\text{sp}}/\text{m}^2\text{g}^{-1}$ is given beside each set of data). Solid lines are results of linear least-squares fits to the points. The dashed lines indicate the temperature dependence of the longitudinal relaxation rates in pure water (10% ^2H). The ordinate scales refer to the dashed lines; other data are displaced as indicated by numbers in parentheses.
- Fig. 2. Concentration dependence of the ^{17}O (34.6 MHz) and ^2H (39.1 MHz) normalized excess relaxation rates in aqueous (10% ^2H , 0.5% ^{17}O) dispersions of colloidal silica. Squares refer to $A_{\text{sp}} = 226 \text{ m}^2\text{g}^{-1}$, 23 $^{\circ}\text{C}$; circles to $A_{\text{sp}} = 68 \text{ m}^2\text{g}^{-1}$, 23.3 $^{\circ}\text{C}$. Open symbols refer to R_1 , filled symbols to R_2 . R_F values were obtained from the dashed lines of fig. 1. Solid lines correspond to linear least-squares fits to the data (including the origin). Error bars as shown apply to all data points, including the origin. Note the different scale for the $R_2(^2\text{H})$ data.
- Fig. 3. Particle size dependence of the ^{17}O normalized excess relaxation rates in aqueous (10% ^2H , 0.5% ^{17}O) dispersions of colloidal silica. The data have been normalized with respect to sol concentration and specific area (see text), as well as to the relaxation rate of pure water (cf. Fig. 1). The samples are described in table 1. Circles refer to experiments at 34.6 MHz, 3.7 $^{\circ}\text{C}$; squares to 34.6 MHz, 26.5 $^{\circ}\text{C}$; and triangles to 49.0 MHz, 26.7 $^{\circ}\text{C}$. Open symbols refer to R_1 , filled symbols to R_2 . Error bars vary for different sols due to the normalization procedure; for a given sol the uncertainty is independent of temperature and resonance frequency.

Fig. 4. As figure 3, but for the ^2H relaxation at 39.1 MHz. Symbols refer to the same temperatures as in figure 3. Note the different scale for the $R_2(^2\text{H})$ data.

Fig. 5. Normalized ^{17}O (34.6 MHz) and ^2H (39.1 MHz) relaxation rates versus volume added 2M NaOH to an aqueous (10% ^2H , 0.5% ^{17}O) dispersion of colloidal silica ($A_{\text{sp}} = 133 \text{ m}^2\text{g}^{-1}$, $W_{\text{SiO}_2} = 2.28 \text{ g}$, $W_{\text{H}_2\text{O}} = 3.85 \text{ g}$). Open symbols refer to R_1 , filled symbols to R_2 . The temperature was 22 $^{\circ}\text{C}$. Note the different scale for the $R_2(^2\text{H})$ data.

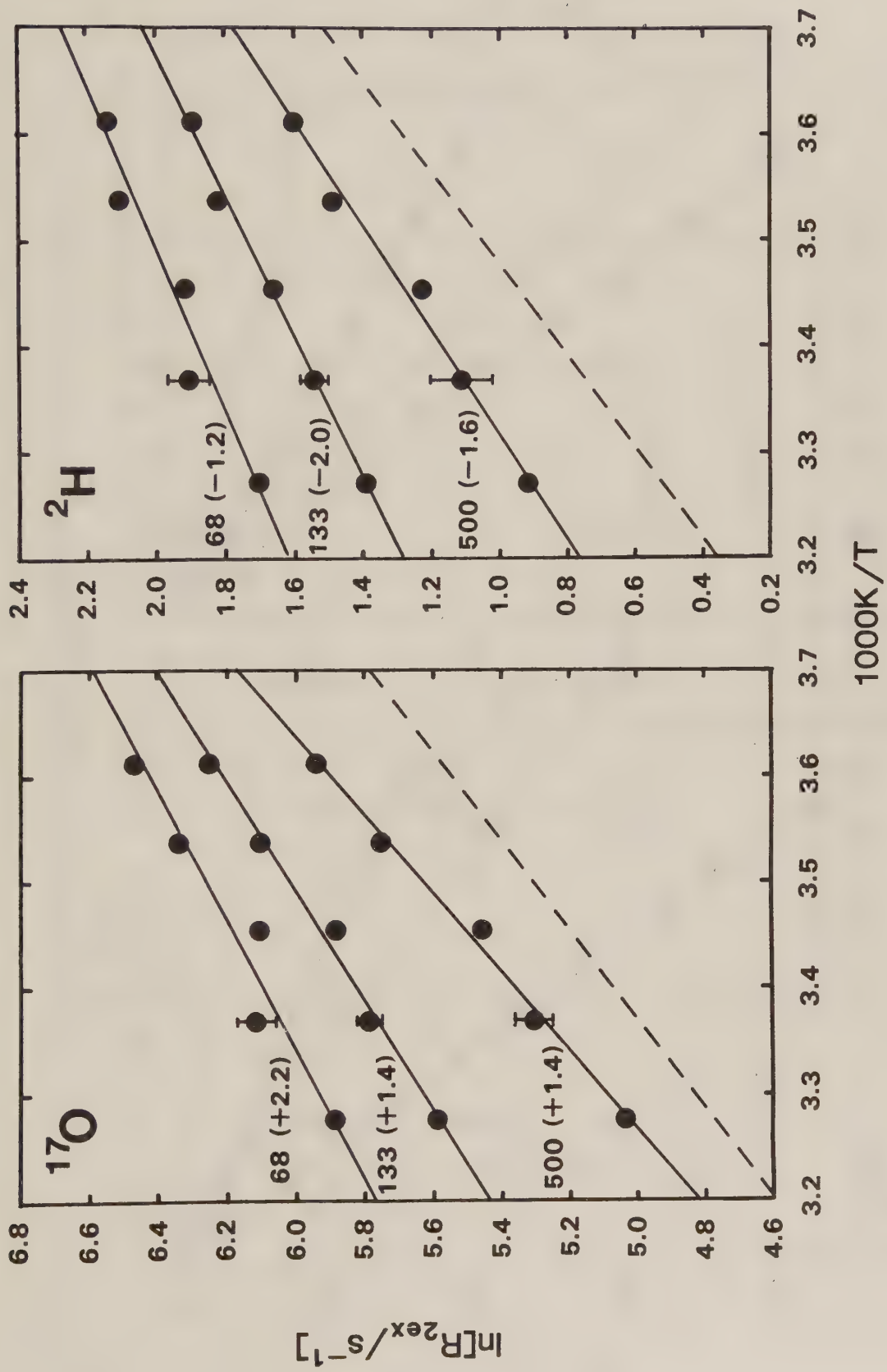


FIGURE 1

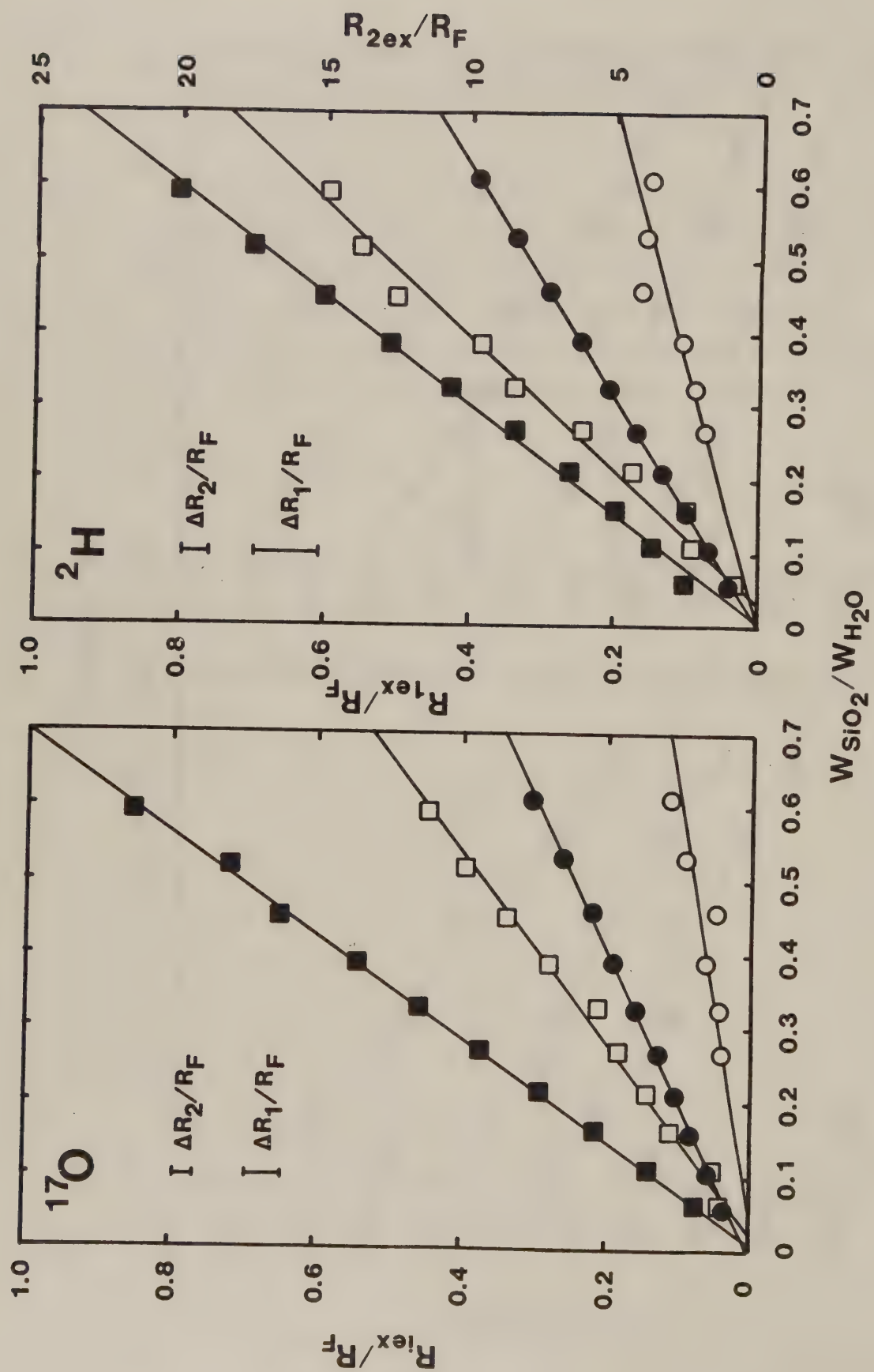


FIGURE 2

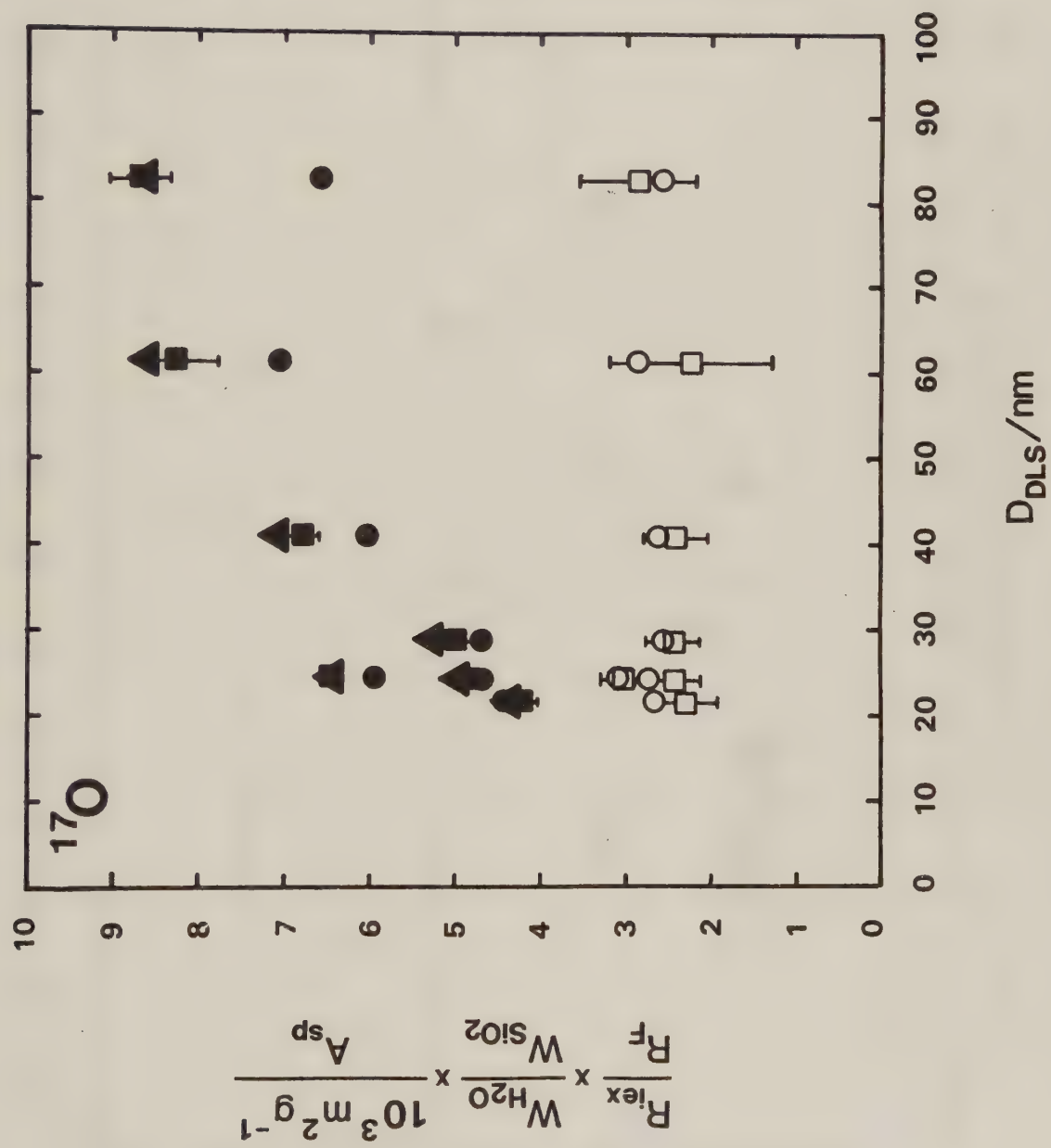


FIGURE 3

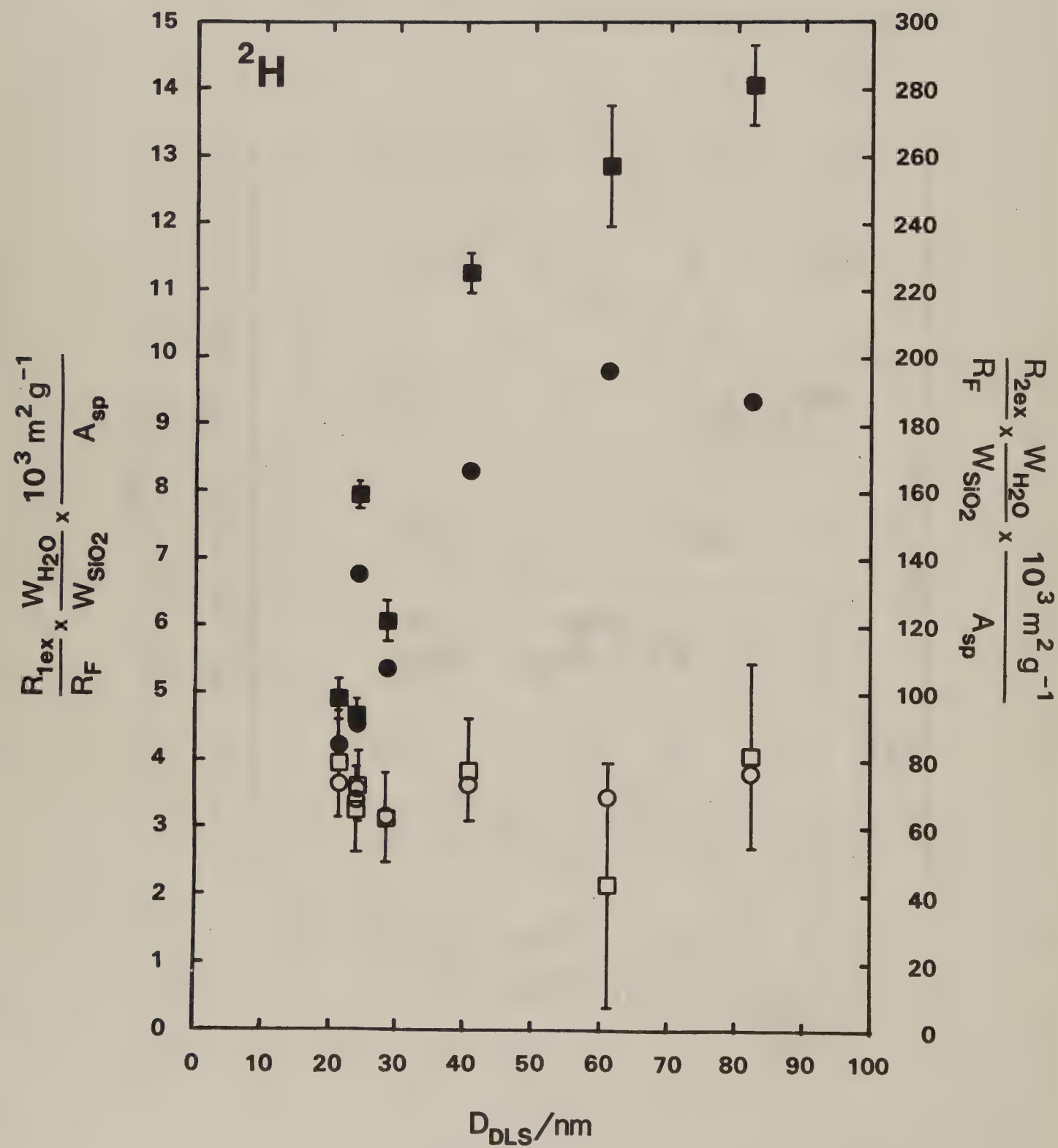


FIGURE 4

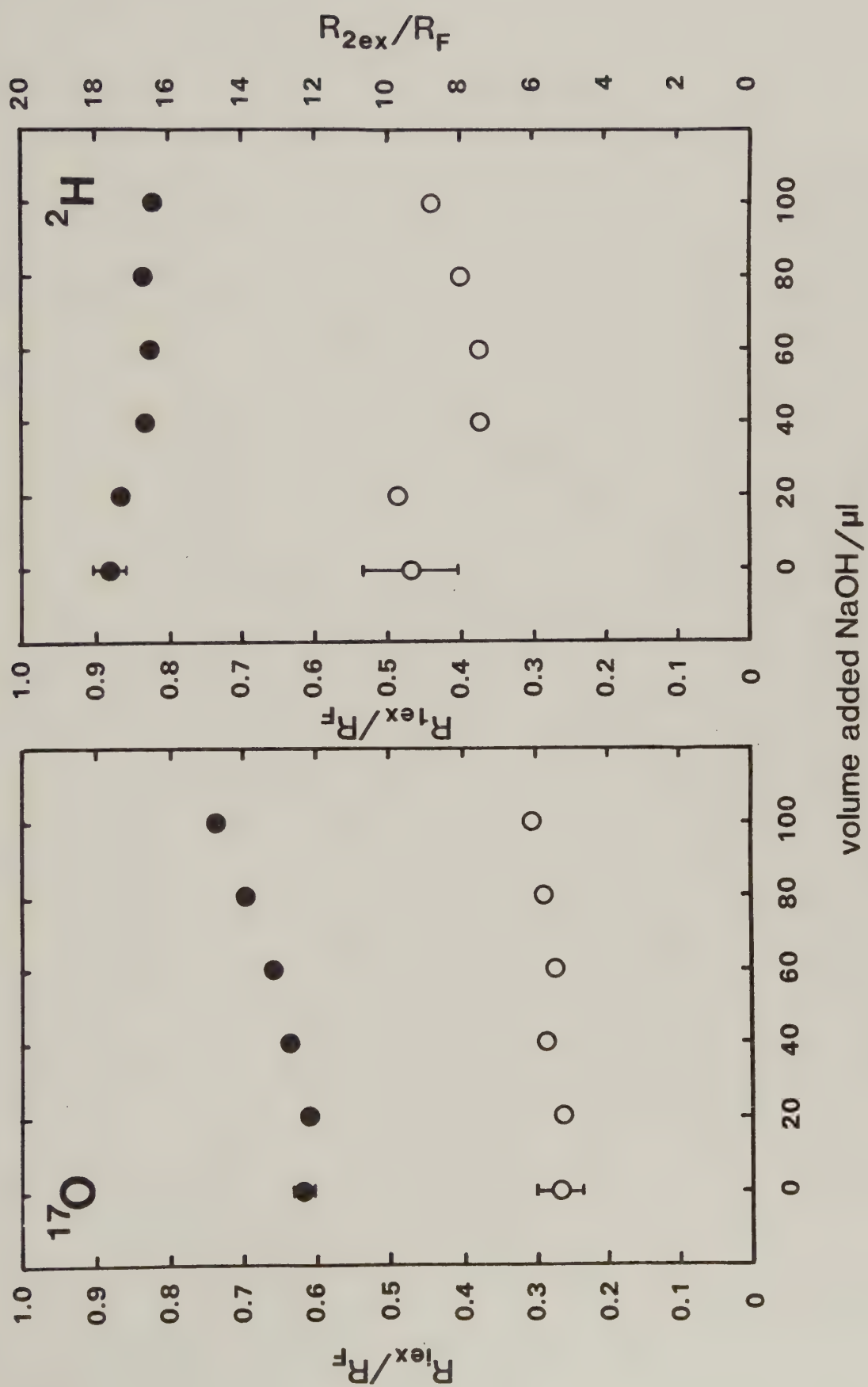


FIGURE 5

VI

WATER SPIN RELAXATION IN COLLOIDAL SYSTEMS

Part 2. ^{17}O and ^2H Relaxation in Protein Solutions.

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ABSTRACT

Oxygen-17 and deutron longitudinal and transverse spin relaxation rates from aqueous protein solutions (mainly lysozyme and hemocyanin) have been measured as a function of temperature, pH and resonance frequency. A detailed comparison of the ^{17}O and ^2H data demonstrates that ^2H relaxation has a dominant contribution which is not seen by ^{17}O . This contribution is, at least partly, due to deutron-exchange with ionizable groups on the protein. Furthermore, we refute two widespread beliefs about solvent spin relaxation in protein solutions, namely that the discrete-exchange model leads to interpretational inconsistencies and that the low-frequency dispersion always reflects protein reorientation.

INTRODUCTION

During the last two decades, spin relaxation of the water nuclei ^1H and ^2H has been used extensively to probe the hydration and molecular dynamics in aqueous protein solutions¹⁻⁶. Recent evidence⁷⁻⁹ of cross-relaxation between the solvent and protein spin systems has made it clear that the vast majority of relaxation studies reported to date, viz., those employing the isotope ^1H , do not provide readily accessible information about the behaviour of water molecules in the investigated systems. The other two magnetic water nuclei, ^2H and ^{17}O , are relaxed efficiently through the intramolecular quadrupole mechanism and are not significantly affected by dipolar cross-relaxation.

Another serious complication in spin relaxation studies of protein solutions is the possibility of chemical exchange of solvent protons (or deuterons) with ionizable groups on the protein molecule. The dramatic effect of such exchange on ^2H relaxation in aqueous solutions of polyelectrolytes containing carboxyl groups is now well documented¹⁰⁻¹³. Very recently, large deuteron exchange effects on ^2H relaxation have been documented also in silica sols¹⁴ (hydroxyl groups) and in surfactant systems¹⁵ (amine groups). It is expected, then, that such exchange effects are present also in solutions of protein molecules with their abundance of prototropic residues.

Only the isotope ^{17}O is immune to the complications of cross-relaxation and exchange with ionizable groups. Hence, only by using

^{17}O relaxation can we be certain, in general, of observing the behaviour of water molecules in macromolecular solutions. Yet, only one systematic ^{17}O relaxation study¹⁶ of protein solutions has so far been reported. In that work, a self-consistent picture of hydration and dynamics in protein solutions was obtained by interpreting the ^{17}O relaxation data in terms of a discrete-exchange model¹⁷ (DEM) with two states ("free" and "bound" water) and a two-step model (TSM)¹⁸ for the orientational time correlation function of the "bound" water. The conclusions from that ^{17}O study deviate in important respects from the conclusions drawn by Koenig, Hallenga and Shporer (KHS) in a paper¹⁹ which, during the last decade, has often³⁻⁶ been taken to indicate the state of the art of NMR as applied to the problem of hydration and water dynamics in protein solutions. The present study was undertaken in the hope of resolving these interpretational controversies.

We have chosen to examine in detail two systems for which extensive ^2H and ^{17}O relaxation data are now available, viz., aqueous solutions of lysozyme (a typical medium-sized protein) and hemocyanin (an exceptionally large protein). (We do not discuss ^1H data, since their interpretation is complicated by cross-relaxation effects.) The ^2H relaxation data of KHS¹⁹, complemented by the new ^2H and ^{17}O data reported here, constitute a critical test of any theoretical model for the interaction and dynamics of water in protein solutions. Our aim is to present the simplest interpretation consistent with this extensive body of relaxation data. Special attention will be given to the following two questions: (1) do the

^2H and ^{17}O relaxation rates report on the same molecular species?, and (2) can the data be described by a two-state DEM?

The paper is organized as follows. After the experimental section, we present a theoretical framework of the minimum complexity required to account for the previously reported^{16,19} frequency dependence of the ^2H and ^{17}O relaxation rates. We then report, in section 4, new relaxation data at variable temperature, pH and frequency, demonstrating that the ^2H and ^{17}O relaxation rates do not monitor the same molecular entities. In section 5 we discuss the origin of the disparity between the ^2H and ^{17}O results. Finally, in section 6, we recapitulate our main conclusions.

The present work constitutes part 2 in a series of three papers on water spin relaxation in colloidal systems. Part 1¹⁴ presents extensive relaxation data from aqueous dispersions of colloidal silica particles - a well-characterized model system which in many respects is simpler than protein solutions. Part 3²⁰ contains a detailed analysis of the origin of the long-time tail in the correlation function, which gives rise to the observed frequency dependence and the inequality of longitudinal and transverse ^{17}O relaxation rates in colloidal systems.

2. EXPERIMENTAL

2.1. MATERIALS

Hemocyanin: α -Hemocyanin from *H. Pomatia* was a kind gift from Dr. L. Zolla.

Dialysis against 0.050 M Tris-HCl, 0.10 M NaCl, pH 9.0 or 0.050 M Tris-HCl, 0.010 M CaCl_2 yielded the 1/10 L ($M_r = 9 \times 10^5$) or 1/1 ($M_r = 9 \times 10^6$) forms of the protein, respectively²¹. Water enriched in ^{17}O and/or ^2H was then added, yielding the final isotopic compositions given below. Solutions were prepared and stored at ca. 4°C . Hemocyanin concentrations were determined spectrophotometrically in 2% borate buffer using $\epsilon^{278} = 14.16\%^{-1} \text{ cm}^{-1}$ ²².

Other proteins. Egg white lysozyme (Sigma, grade 1), Human Plasma Albumin (Kabi Vitrum) and Immunoglobulin G (Kabi Vitrum) were obtained as lyophilizates and were dissolved, without further purification, in water of isotopic composition as specified. The pH was adjusted through additions of HCl(DCl), NaOH(NaOD) or KOD (in the cases of HPA and IGG).

2.2. RELAXATION MEASUREMENTS

^{17}O and ^2H relaxation rates were measured at resonance frequencies ranging from 4 to 56 MHz. Resonance frequencies are specified for each particular experiment. The following spectrometers were used: A Bruker BKr-322s with a Varian V-71 computer (^{17}O and ^2H at the three lowest fields), a modified Varian XL-100-15 (^{17}O at 13.6 MHz), a home-built spectrometer equipped with a 6 T magnet (^{17}O at 34.6 MHz, ^2H at 39.1 MHz), and a Nicolet NIC-360 (^2H at 55.5 MHz). Longitudinal relaxation rates (R_1) were measured by inversion recovery. Oxygen-17 transverse relaxation

rates (R_2) were obtained from the linewidth according to $R_2 = \pi \Delta\nu$, where $\Delta\nu$ is the linewidth at half amplitude of the absorption spectrum, corrected for inhomogeneity broadening (ca. 2 Hz). Deuteron transverse relaxation rates were measured using the Carr-Purcell-Meiboom-Gill pulse sequence. In all experiments, reference solutions of slightly acidic water with known, frequency-independent relaxation rates were measured, and the error estimates given in tables and figures are based on the reproducibility of these measurements. Measurements were in all cases completed within 24 hours, with the exception of the frequency dependence of table 2, which was measured within 48 hours. Separate determinations, one day apart, of the ^{17}O rates at 34.6 MHz (table 2) revealed no time dependence. The temperature was kept constant to $\pm 0.5^\circ\text{C}$ by passage of dry thermostated air or nitrogen through the probe.

2.3. SCALAR RELAXATION

Modulation of the scalar coupling between ^{17}O and ^1H (^2H) through proton (deuteron) exchange will, in general, contribute to the transverse relaxation of water nuclei²³. The respective scalar relaxation rates depend on the exchange rates, being largest around neutral pH where proton (deuteron) exchange is slow, and on the isotopic composition of the water. Since at low concentrations solutes containing prototropic residues can only enhance the effective proton exchange rates, the scalar relaxation in pure water of the same isotopic composition and at the same pH provides an upper limit to the scalar relaxation of a given protein

solution. It was thus found that scalar contributions^{24,25} in all cases are completely negligible compared to reported ^{17}O and ^2H excess transverse rates, with the possible exception of ^{17}O in hemocyanin, where scalar relaxation may be of the order of the reported experimental uncertainties.

2.4. NONEXPONENTIAL RELAXATION

The relaxation of ^{17}O ($I=\frac{5}{2}$) is strictly exponential only under extreme narrowing conditions, i.e. when $R_2 = R_1$ and the relaxation is independent of resonance frequency. However, when R_2/R_1 is of order one, the relaxation will be effectively exponential, and approximate analytical expressions for the measured rates can be used³⁸. The errors in such a treatment can be evaluated³⁸, and in the present case they are significant only for $R_2(^{17}\text{O})$ of hemocyanin 1/1, amounting to less than 3.5% at the highest frequencies of table 2 and less than 5.5% in the temperature dependence of figure 1.

3. THEORETICAL MODEL FOR WATER SPIN RELAXATION IN PROTEIN SOLUTIONS

The central experimental observations^{16,19,26} common to previous ^2H and ^{17}O relaxation studies of protein solutions are:

- (1) A frequency dependence around a characteristic frequency $\omega_c \equiv \tau_c^{-1}$ and a consequent inequality of the longitudinal (R_1) and transverse (R_2) relaxation rates: $R_1 < R_2$ for $\omega \gtrsim \omega_c$.

- (2) A nonzero, frequency-independent contribution to R_1 at high frequencies $\omega \gg \omega_c$.

The simplest possible expressions for the relaxation rates that are compatible with these observations and with basic relaxation theory²⁷ are

$$R_1(\omega_0) = \alpha + \beta \left[\frac{0.2}{1+(\omega_0\tau_c)^2} + \frac{0.8}{1+(2\omega_0\tau_c)^2} \right] \quad (1a)$$

$$R_2(\omega_0) = \alpha + \beta \left[0.3 + \frac{0.5}{1+(\omega_0\tau_c)^2} + \frac{0.2}{1+(2\omega_0\tau_c)^2} \right] \quad (1b)$$

The simplest interpretation of the parameters α and β in eq. (1) is in terms of a two-state DEM¹⁷ in the fast-exchange limit, according to which the observed rates are simple averages over the states. In terms of the water fractions (P_B , P_F) and the intrinsic relaxation rates (R_F , R_{iB})

$$R_1(\omega_0) = P_F R_F + P_B R_{1B}(\omega_0) \quad (2a)$$

$$R_2(\omega_0) = P_F R_F + P_B R_{2B}(\omega_0) \quad (2b)$$

As usual, subscripts F and B denote "free" and "bound" states, the former being defined by the state of the sample with the protein component removed.

KHS attempted an interpretation of their ^2H dispersion data which amounts to setting $\alpha = P_F R_F$. This implies that the dynamics in the B state can be described by a single correlation time. However, as concluded by KHS, such an interpretation has two deficiencies:

- (1) It cannot explain the observation that $\alpha > R_F$. (This fact is most clearly seen from the high-frequency data of ref. 16. Cf. also the fits to the data of KHS in Figs. 3 and 4.)
- (2) It implies that the B state comprises an unphysically small number of water molecules, e.g., 3 per molecule of lysozyme¹⁹.

A way out of these difficulties can be found by recognizing that a water molecule near a protein surface, although it still reorients nearly as fast as in the bulk¹⁶, necessarily senses an anisotropic environment. A theory for relaxation in locally anisotropic systems, with special emphasis on water nuclei, has been presented¹⁸ and applied to various systems^{12,14,16,28}. We emphasize that this so-called TSM theory is formulated in very general, essentially model-independent terms, and that it may be combined with specific models for the underlying interactions and dynamics²⁰. Using the TSM theory, then, to get expressions for R_{1B} and R_{2B} in eq. (2), we can make the following identifications in eq. (1):

$$\alpha = \frac{3\pi}{10} \frac{2I+3}{I^2(2I-1)} \chi^2 [P_F(1 + \frac{\eta^2}{3})\tau_F + P_B(1 + \frac{\eta^2}{3} - A^2)\tau_{Bf}] \quad (3a)$$

$$\beta = \frac{3\pi^2}{10} \frac{2I+3}{I^2(2I-1)} P_B(A\chi)^2\tau_{Bs} \quad (3b)$$

$$\tau_c = \tau_{Bs} \quad (3c)$$

In eq. (3), I is the nuclear spin quantum number, χ is the quadrupole coupling constant and η is the asymmetry parameter of the field gradient tensor. The fast, local reorientation of water molecules is described by the effective correlation times τ_F and τ_{Bf} in the respective states. The observed slow dynamics are described by an effective correlation time τ_{Bs} (cf. below). The residual anisotropy, A , is related to the order parameters S_0^2 and S_2^2 , as defined in ref. 18, through

$$|A(^2H)| = 0.10 S_0^2 + 0.41 S_2^2 \quad (4a)$$

$$|A(^{17}O)| = 0.03 S_0^2 + 0.80 S_2^2 \quad (4b)$$

Eq. (3a) shows that if, as expected, $A^2 \ll 1$ and $\tau_{Bf} > \tau_F$, then $\alpha > R_F$, as observed. Moreover, from eq. (3b) we see that what was interpreted by KHS as the fraction P_B of water molecules in the B state is actually $P_B A^2$, where, typically, A^2 is of the order 10^{-3} . The deduced number of "bound" water molecules thus increases by a factor of order 10^3 .

On the basis of their 2H dispersion data, interpreted in terms of eq. (1a) with $\alpha = P_F R_F$, KHS were led to reject the DEM because:

- (1) It leads to an unphysically small number of water molecules in the B state.
- (2) It generates inconsistencies connected with the lifetime of a water molecule in the B state.

We have just seen that the first of these two paradoxes can be removed by using the DEM in combination with the TSM theory for spin relaxation in locally anisotropic systems¹⁸. We shall now show that the TSM resolves also the second paradox.

After local averaging of the quadrupole coupling by the, slightly restricted, reorientational motions at the protein surface there remains a residual coupling, A_χ . Other dynamical processes in the system cause A_χ to fluctuate (with zero mean) at a comparatively slow rate, thus giving rise to a long-time tail in the correlation function. A detailed analysis²⁰ reveals that this tail decays nearly exponentially (as implied by eq. (1)) with a correlation time τ_{Bs} given by

$$\frac{1}{\tau_{Bs}} = \frac{1}{\tau_{lat}} + \frac{1}{\tau_{rot}} + \frac{1}{\tau_{res}} \quad (5)$$

where τ_{lat} characterizes the lateral diffusion of the spin-bearing species within the B state, τ_{res} is the mean residence time in that state, and τ_{rot} is the rotational correlation time of the protein molecule. Eq. (5) provides a lower limit for τ_{res}

$$\tau_{Bs} \leq \tau_{res} \quad (6)$$

while the fast-exchange criterion^{17,29} provides an upper limit, which, for a reasonably dilute protein solution, is given by

$$\tau_{res} \ll R_{2B}^{-1}(\omega_0) \leq R_{1B}^{-1}(\omega_0) \quad (7a)$$

If it can be demonstrated that fast-exchange conditions apply at frequencies below the dispersion region, then the following more restrictive condition applies

$$\tau_{res} \ll R_B^{-1}(0) \quad (7b)$$

Because of the much larger quadrupole coupling, ¹⁷O relaxation will furnish the lowest upper bound.

In Table 1 we present lower and upper bounds on τ_{res} , as given by eqs. (6) and (7b), for the three systems studied by KHS. The model considered by KHS is one with rigidly (or irrotationally) bound water molecules in the B state. This limit is obtained by setting $A^2 = 1 + \frac{\eta^2}{3}$ in eq. (3). The resulting upper bounds, calculated on the basis of ²H or ¹⁷O relaxation, are given in Table 1. These bounds (the ¹⁷O upper bound for hemocyanin was not used since fast exchange for ¹⁷O in this system had not been established by KHS) led KHS to reject the DEM, because the bounds on τ_{res} appeared too restrictive, and because the one order of magnitude difference in τ_{res} between lysozyme and hemocyanin was considered unphysical.

However, if we give up the notion of rigid binding and instead use the TSM with a typical $|A| = 0.05$, we avoid the difficulties that forced KHS to reject the exchange model (cf. Table 1). Our conclusion, then, is that we must reject the rigid binding model - not the DEM*.

In the following we shall discuss all relaxation data in terms of the DEM/TSM theory outlined in this section. We thus distinguish two (or more) discrete states for water in protein solutions; a "free" state, wherein the water is unaffected by protein molecules, and a "bound" state, in which the water has retained most of its reorientational freedom while having a slight orientational bias with respect to the protein surface.

* In an early paper³⁰, Koenig and Schillinger consider one particular case of restricted local motion, namely rotation of the water molecule about an axis which is fixed with respect to the protein. They then proceed to show that for the case of a uniform distribution of angles α between the rotational axis and the proton-proton vector of the water molecule, $[A(^1H)]^2 = \frac{1}{5}$. This leads them to conclude that local motions of the type considered cannot resolve the inconsistencies (absurdly low "hydration numbers" and too restrictive bounds on the water lifetimes). This conclusion is invalid for two reasons:

4. COMPARISON OF ^2H AND ^{17}O RELAXATION

An assumption underlying all previously published interpretations of ^2H spin relaxation rates from protein solutions is that the data report exclusively on the behaviour of deuterons residing in water molecules. In other words, one assumes that the deuteron exchange between solvent and ionizable side chains on the protein is in the slow-exchange limit. This assumption may be tested by comparing ^2H and ^{17}O relaxation rates from the same system, knowing that the ^{17}O rate necessarily reflects the behaviour of the solvent. In making such a comparison, it must be borne in mind that, even in the absence of deuteron exchange, the ^2H and ^{17}O relaxation do not necessarily monitor the same water molecules. Because of the much stronger quadrupole coupling for ^{17}O , the fast-exchange condition is more restrictive for ^{17}O than it is for ^2H (cf. eq. (7b)). It is thus conceivable that a number of water molecules exchange between the protein surface and the bulk at rates such that they contribute to ^2H relaxation (fast exchange) but not to ^{17}O relaxation (slow exchange). Finally, even if they monitor the same water mole-

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- (1) The result does not carry over to $A(^2\text{H})$ and $A(^{17}\text{O})$ [cf. eq. (4)]
 - (2) The calculation of Koenig and Schillinger is incorrect: they average the square of $\frac{1}{2}(3\cos^2\alpha-1)$, rather than squaring the average. (For an isotropic distribution of rotation axes, as assumed by Koenig and Schillinger, the residual anisotropy is zero.)

Yet, this factor $\frac{1}{5}$ is used as an argument against models with non-rigidly bound water molecules in subsequent papers^{4,19}.

cules, the ^2H and ^{17}O rates will not, in general, scale as they do in pure water. This is so because the relative contributions from fast and slow motions to the relaxation rates, i.e. the α - and β -terms in eq. (1), will be different for the two nuclei since $A(^2\text{H}) \neq A(^{17}\text{O})$ (cf. eq. (4)). In order to ascertain whether the two nuclei do in fact report on the same species we present in this section new ^{17}O and ^2H relaxation data, mainly from aqueous solutions of lysozyme and hemocyanin, as a function of temperature, pH and frequency.

4.1. TEMPERATURE DEPENDENCE

The purpose of a study of the temperature dependence of the ^2H and ^{17}O relaxation rates is twofold. Firstly, if "bound" and "free" water molecules exchange rapidly, as assumed in eqs. (2) and (7), then an Arrhenius plot of $\ln R_{2\text{ex}}$ versus $1/T$ should exhibit a positive slope. (The excess relaxation rates are conventionally defined as $R_{i\text{ex}} \equiv R_i - R_F$, $i = 1, 2$.) Secondly, the apparent activation energies obtained from the slope in the Arrhenius plot contains information about the molecular processes that induce spin relaxation. ^{17}O relaxation rates from protein solutions in the temperature range $0 - 40^\circ\text{C}$ yield activation energies of $15 - 20 \text{ kJ mol}^{-1}$ (see Fig. 1 and ref. 16), which may be compared with ca. 20 kJ mol^{-1} obtained from pure water in the same temperature range³¹. One therefore expects that the contributions from fast and slow motions of rapidly exchanging water molecules to the relaxation rates in protein solutions should have closely similar activation energies. The fact that these two contributions are weighted

differently for ^2H and ^{17}O should thus not affect the temperature dependence, i.e. the ^2H and ^{17}O relaxation rates should have nearly the same activation energies.

Fig. 1 shows the temperature dependence of the ^{17}O and ^2H excess transverse relaxation rates in lysozyme and hemocyanin solutions. In the case of lysozyme, both nuclei indicate fast-exchange conditions with identical (within the experimental uncertainty) activation energies. For hemocyanin, however, the two nuclei give qualitatively different results. This finding implies that there is a large additional contribution to the ^2H relaxation which is not seen by ^{17}O . The additional contribution may be associated with protein-bound deuterons and/or with water molecules which exchange slowly on the ^{17}O (but not on the ^2H) timescale. Furthermore, it appears from Fig. 1 that the rate of exchange of the additional species is in the intermediate regime, i.e. of the same order as the intrinsic ^2H relaxation rate for this species.

4.2. pH DEPENDENCE

The observation of an identical temperature dependence in the ^2H and ^{17}O relaxation rates is a necessary, but not sufficient, condition to conclude that the two nuclei monitor the same species. An additional test is provided by the pH dependence of the relaxation rates. If the two nuclei report on the same species, then the ^2H and ^{17}O rates should vary with pH in qualitatively the same way. (Quantitative differences may occur because of the different weighting of the fast and slow contributions for the two nuclei.)

Fig. 2 shows the pH dependence of the ^{17}O and ^2H longitudinal and transverse relaxation rates in lysozyme solutions. The dramatic increase in the ^2H transverse rate at low pH, completely absent for ^{17}O , suggests a large contribution from deuteron exchange with carboxylic side chains on the protein. (The pH dependence in hemocyanin solutions is complicated by changes in conformation and aggregation²¹ and was therefore not studied.)

4.3. FREQUENCY DEPENDENCE

If the ^2H and ^{17}O resonances are due exclusively to nuclei residing in the same water molecules, then the only parameters in eq. (3) that depend on the identity of the observed nucleus are I , χ , A and η . Since $A^2 \ll 1$ we may safely neglect A^2 in eq. (3a) to obtain

$$\frac{\alpha(^{17}\text{O})}{\alpha(^2\text{H})} = \frac{8}{125} \frac{\chi^2(^{17}\text{O}) [1 + \frac{1}{3}\eta^2(^{17}\text{O})]}{\chi^2(^2\text{H}) [1 + \frac{1}{3}\eta^2(^2\text{H})]} = \frac{R_F(^{17}\text{O})}{R_F(^2\text{H})} \quad (8a)$$

$$\frac{\beta(^{17}\text{O})}{\beta(^2\text{H})} = \frac{8}{125} \left[\frac{\chi(^{17}\text{O})A(^{17}\text{O})}{\chi(^2\text{H})A(^2\text{H})} \right]^2 \quad (8b)$$

Note that the ratios of α , β and R_F in eq. (8) refer to ^2H and ^{17}O data on the same system, i.e., the temperature, the solvent isotope composition, etc. all have to be the same.

In all reported cases, where the ^2H and ^{17}O resonances are due to the same water molecules, the ratio of the residual anisotropies, as deduced from the observed quadrupole splittings, is, to within 20%, given by¹⁸

$$\left| \frac{A(^{17}\text{O})}{A(^2\text{H})} \right| = 2 \quad (9)$$

This invariance of $A(^{17}\text{O})/A(^2\text{H})$ with respect to the chemical nature of the interface is in all likelihood a consequence of the nearly linear dependence of eq. (4a) and (4b) for small S_0^2/S_2^2 ratios^{18,32,33}, rather than a physical property common to all the investigated systems. It is thus reasonable to assume that eq. (9) holds also for the protein solutions under discussion.

Table 2 shows ^2H and ^{17}O relaxation rates from aqueous hemocyanin solutions obtained at several resonance frequencies. The inequality of the longitudinal and transverse rates together with the weak frequency dependence in the investigated range, indicate that the major step in the dispersion occurs at lower frequencies, corresponding to $\tau_c = \omega_c^{-1} \gtrsim 4 \times 10^{-8}$ s. According to eq. (1) we then have approximately

$$\alpha = R_1 \quad (10a)$$

$$\beta = \frac{10}{3} (R_2 - R_1) \quad (10b)$$

A slightly more accurate procedure to obtain α and β is to least-squares fit eq. (1) to the data of Table 2. The results of this procedure are given in Table 3, which also includes results for three other proteins. The lysozyme data in Table 3 were calculated from the relaxation rates in Fig. 2 using eq. (10) and the ranges given for the α - and β -ratios correspond to their variation with pH in the investigated range. The albumin and immunoglobulin α - and β -values were calculated in the same way from new relaxation data obtained under the specified conditions. The use of eq. (10) is justified since the frequencies of Table 3 are above the main dispersion^{16,26} in all cases. (Eq. (10b) actually remains a good approximation even within the high-frequency part of the dispersion region.)

The experimental data in Table 3 should be compared with the theoretical α - and β -ratios, calculated from eq. (8) and (9) using data from Table I of ref. 18, and based on the assumption that the ^2H and ^{17}O resonances monitor the same molecular species. The agreement of the $\alpha(^{17}\text{O})/\alpha(^2\text{H})$ ratio is not a critical test of this assumption since, for the larger proteins, α reflects mainly bulk water (F state) properties. More significant is the fact that the experimental $\beta(^{17}\text{O})/\beta(^2\text{H})$ ratios in all cases are an order of magnitude smaller than the theoretical ratio. This leads us to conclude that, in all protein solutions investigated here, there is an additional contribution to ^2H relaxation which is not present in ^{17}O relaxation. In the following section we discuss the origin of this additional contribution.

5. DISCUSSION

The data presented in the previous section show unequivocally that, in all investigated cases, ^2H and ^{17}O relaxation from aqueous protein solutions do not report on the same molecular species. Instead it was found that the ^2H relaxation has an additional contribution (not present in the ^{17}O relaxation), which we ascribe to protein-bound deuterons and/or to a class of water molecules which exchange slowly on the ^{17}O timescale. If this additional contribution may be associated with a reasonably uniform relaxational state, then it might be appropriate to describe the ^2H data in terms of a three-state DEM. In the case of lysozyme, for which fast-exchange conditions have been established (see Fig. 1), the longitudinal ^2H relaxation rate is then given by a straightforward generalization of eq. (1) as

$$R_1(\omega_0) = \alpha + \beta_B f_1(\omega_0 \tau_{BS}) + \beta_P f_1(\omega_0 \tau_{PS}) \quad (11)$$

where f_1 is the bracketed frequency-function in eq. (1a). In eq. (11) subscript B denotes the relaxational state (or species) which is seen by ^2H as well as by ^{17}O and subscript P denotes the additional state observed only in ^2H relaxation. The interpretation of α , β_B and β_P in terms of the TSM theory is given by an obvious generalization of eq. (3) and we assume that the slow correlation times τ_{BS} and τ_{PS} may each be decomposed as in eq. (5). With this interpretation of the parameters, eq. (11) is valid if τ_{res}^B and τ_{res}^P are long compared to τ_{rot} or if the $B \leftrightarrow P$ exchange is much slower than the $F \leftrightarrow B$ exchange.

KHS have measured the entire low-frequency longitudinal ^2H relaxation dispersion in lysozyme solution¹⁹. Fig. 3 shows the result of fitting eq. (1a) to their data. Clearly, the ^2H dispersion does not, by itself, force us to invoke a three-state model. However, the pH dependence (Fig. 2) and the $\beta(^{17}\text{O})/\beta(^2\text{H})$ ratio (Table 3) can be understood only if a third state contributes to the ^2H relaxation. In terms of the three-state model, eq. (11), the single-correlation-time dispersion in Fig. 3 suggests that the slow correlation times are equal: $\tau_{\text{Bs}} = \tau_{\text{Ps}}$. This interpretation explains why different nuclei yield (within uncertainties) the same activation energies for $R_{2,\text{ex}}$ (see Fig. 1) and the same slow correlation times: 19 ns (^{17}O in H_2O , 27°C)¹⁶, 17 - 24 ns (^1H in H_2O , 22°C)²⁶ and 35 - 37 ns (^1H and ^2H in 72% $^2\text{H}_2\text{O}$, 22°C)²⁶. The magnitude of the experimentally deduced slow correlation time suggests that $\tau_{\text{Bs}} = \tau_{\text{Ps}} = \tau_{\text{rot}}$, which, according to eq. (5), implies that the correlation times $\tau_{\text{res}}^{\text{B}}$, $\tau_{\text{res}}^{\text{P}}$ and τ_{lat} are all longer than the rotational correlation time of the protein.

Fig. 4 shows fits of eq. (1a) and (11) to longitudinal ^2H relaxation dispersion data from aqueous hemocyanin solution reported by KHS¹⁹. In contrast to the case of lysozyme, inclusion of a third state leads to a significant improvement of the dispersion fit for hemocyanin. If the interpretation of the B state as that seen also by ^{17}O is correct, then the ratio $\beta(^{17}\text{O})/\beta_{\text{B}}(^2\text{H})$ should agree with the theoretical estimate of 230 (cf. Table 3). Using $\beta_{\text{B}}(^2\text{H})$ from the three-state fit in Fig. 4 and $\beta(^{17}\text{O})$ from Table 3 and correcting for differences in protein concentration and solvent isotope composition we get $\beta(^{17}\text{O})/\beta_{\text{B}}(^2\text{H}) = 130$, reasonably close to the theoretical value. (A possible source of the remaining discrepancy is the fact that the ^{17}O data were obtained at pH 8.93 and the ^2H data at pH 7.0).

Reasonable agreement is obtained also for the slow correlation times:

$\tau_{BS}(^2\text{H}) = 150 \text{ ns}$ (from the three-state fit in Fig. 4) and $\tau_{BS}(^1\text{O}) = 60 \pm 25 \text{ ns}$ (from a two-state fit to ^1O data in Table 2.)

From what has been said it follows that the main dispersion step in Fig. 4 can be ascribed to the additional P state, which contributes only to ^2H relaxation. At present, we cannot determine whether this contribution derives from protein-bound deuterons or from water molecules that exchange slowly on the ^1O timescale. It should also be noted that the very weak temperature dependence of $R_{2,\text{ex}}(^2\text{H})$ (see Fig. 1) may indicate that the exchange with the P state is not fast with respect to R_{2P} . If this is the case, eq. (11) is not strictly valid.

In an isotropic protein solution, no correlation time is longer than the (longest) rotational correlation time, τ_{rot} , of the protein molecule. However, in the presence of other motions, such as translational diffusion of water molecules²⁰, the longest observed correlation time may well be much shorter than τ_{rot} . This is clearly the case for ^1O in solutions of hemocyanin 1/1. In the case of ^2H , it has been argued²⁶ that the measurements of relaxation dispersion is a general method for determining τ_{rot} . However, the value of $\tau_{PS} = 1.1 \mu\text{s}$ deduced for hemocyanin 1/1 (see Fig. 4) is nearly an order of magnitude shorter than the largest rotational correlation time³⁵ for a solid spheroid-cylinder of length 38 nm and diameter 35 nm³⁶: $\tau_{\text{rot}} = 7 - 10 \mu\text{s}$ depending on the eccentricity of the end caps. We conclude then, that an interpretation

of the (possibly pH dependent) low-frequency dispersion of the ^2H relaxation in hemocyanin 1/1 solutions is not straightforward.

In a recent study³⁷, Bryant and Jarvis report ^1H longitudinal relaxation dispersions in concentrated lysozyme solutions with H_2O and $\text{Me}_2\text{SO}/\text{D}_2\text{O}$ as solvents. The finding that the water ^1H and Me_2SO ^1H dispersions are qualitatively similar is taken to "clearly demonstrate that explanations of magnetic relaxation need not invoke solvent proton exchange as a mechanism for the dynamical coupling of the macromolecule to the solvent." Whether or not a low-frequency dispersion in the solvent spin relaxation can be induced in protein solutions also in the absence of proton (or deuteron) exchange with the protein is not the issue in the present work. (That this is indeed the case is, however, clear from this and other^{12,14,16} water ^{17}O relaxation studies.) Rather, we ask whether ^2H (and thus also ^1H) relaxation from protein solutions is significantly affected by deuteron (or proton) exchange with the protein. At least for lysozyme solutions, the answer is in the affirmative.

6. CONCLUSIONS

A detailed comparison of ^{17}O and ^2H spin relaxation rates, at variable temperature, pH and resonance frequency, from aqueous protein solutions has led us to the following conclusions.

- (1) In all investigated cases the contribution from slow motions to the ^2H relaxation rates is dominated by a contribution, not seen by ^{17}O , from protein-bound deuterons exchanging with solvent deuterons and/or from water molecules which exchange slowly on the ^{17}O time-scale. A contribution from protein-bound deuterons is most easily identified by comparing the pH dependence of ^2H and ^{17}O relaxation, as reported here for lysozyme.
- (2) The available ^{17}O relaxation data can be adequately interpreted in terms of a discrete-exchange model (DEM) with fast exchange of water molecules between two relaxational states. In the case of ^2H relaxation, a third state must be added. When combined with the two-step model (TSM), the DEM does not generate any inconsistencies in the deduced exchange rates.
- (3) The characteristic frequency in the relaxation dispersion does not necessarily report on protein reorientation. Thus, for ^{17}O in hemocyanin 1/1, the deduced slow correlation time corresponds to dynamics two orders of magnitude faster than protein reorientation.

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Table 1. Bounds on the mean residence time

System	Lower bound on τ_{res}		Upper bound on τ_{res}		
	$\tau_{\text{Bs}}/\text{s}^{\text{a}}$	Nucleus	$ A $	$R_{\text{B}}^{-1}(0)\text{s}^{\text{b}}$	Nucleus
Lysozyme (4°C)	$9.0 \cdot 10^{-8}$	^2H	1	$1.5 \cdot 10^{-5}$	^2H
			1	$2.6 \cdot 10^{-7}$	^{17}O
			0.05	$1.1 \cdot 10^{-4}$	^{17}O
Lysozyme (22°C)	$3.5 \cdot 10^{-8}$	^2H	1	$3.9 \cdot 10^{-5}$	^2H
			1	$6.8 \cdot 10^{-7}$	^{17}O
			0.05	$2.7 \cdot 10^{-4}$	^{17}O
Hemocyanin 1/1 (25°C)	$9.3 \cdot 10^{-7}$	^2H	1	$1.5 \cdot 10^{-6}$	^2H
			1	$2.6 \cdot 10^{-8}$	^{17}O
			0.05	$1.0 \cdot 10^{-5}$	^{17}O

^aTaken from table 1 of ref. 19.

^bCalculated from $R_{\text{B}}(0) = \frac{3\pi^2}{10} \frac{2I+3}{I^2(2I-1)} (A\chi)^2 \tau_{\text{Bs}}$ [cf. eqs. (1)-(3)] with

$\chi(^2\text{H}) = 0.222 \text{ MHz}$, $\chi(^{17}\text{O}) = 6.66 \text{ MHz}^{18}$ and assuming $\tau_{\text{Bs}}(^2\text{H}) = \tau_{\text{Bs}}(^{17}\text{O})$.

Table 2. ^2H and ^{17}O relaxation rates from aqueous (23% ^2H , 2.3% ^{17}O) hemocyanin solutions^a

Nucleus	ν_0/MHz	Hemocyanin 1/1 ^b		Hemocyanin 1/10 ^c	
		R_1/s^{-1}	R_2/s^{-1}	R_1/s^{-1}	R_2/s^{-1}
^2H	4.605	2.49 ± 0.12		2.61 ± 0.13	
	9.210	2.45 ± 0.12		2.41 ± 0.12	
	13.816	2.29 ± 0.11		2.30 ± 0.11	
	39.141	2.18 ± 0.11	7.5 ± 0.8	2.17 ± 0.11	4.1 ± 0.4
	55.537	1.97 ± 0.10	8.3 ± 0.8	1.94 ± 0.10	4.9 ± 0.5
^{17}O	4.067	201 ± 10		189 ± 9	
	8.134	184 ± 9		184 ± 9	
	12.201	179 ± 9		174 ± 8	
	13.565		239 ± 5		210 ± 4
	34.566	163 ± 8	229 ± 5	160 ± 8	206 ± 4

^aThe temperature was 25°C, except for the ^2H data at 55.537 MHz where it was 28°C. In pure water with 23% ^2H at 25°C the relaxation rates are³⁴
 $R_F(^2\text{H}) = 2.04 \text{ s}^{-1}$ and $R_F(^{17}\text{O}) = 151 \text{ s}^{-1}$.

^b11.0 mg/ml, pH 8.93

^c11.4 mg/ml, pH 8.91

Table 3. α and β values derived from ^2H and ^{17}O relaxation data from aqueous protein solutions

Protein	Conditions	ν_0/MHz	$\alpha(^2\text{H})/\text{s}^{-1}$	$\alpha(^{17}\text{O})/\text{s}^{-1}$	$\frac{\alpha(^{17}\text{O})}{\alpha(^2\text{H})}$	$\beta(^2\text{H})/\text{s}^{-1}$	$\beta(^{17}\text{O})/\text{s}^{-1}$	$\frac{\beta(^{17}\text{O})}{\beta(^2\text{H})}$
Hemocyanin 1/1	11.0 mg/ml, pH 8.93,	4-55	2.15 $\pm$ 0.11	169 $\pm$ 8	79 $\pm$ 8	19.0 $\pm$ 3.8	205 $\pm$ 10	11 $\pm$ 3
	23% ^2H , 2.3% ^{17}O , 25°C.							
Hemocyanin 1/10	11.4 mg/ml, pH 8.91,	4-55	2.11 $\pm$ 0.11	168 $\pm$ 8	79 $\pm$ 8	7.8 $\pm$ 1.6	125 $\pm$ 6	16 $\pm$ 6
	23% ^2H , 2.3% ^{17}O , 25°C							
Lysozyme	82-84 mg/ml, pH 2.2-	34.6			70-79 ^a			23-63 ^a
	5.5, 100% ^2H , 0.5% ^{17}O , 23°C							
Human plasma albumin	28 mg/ml, pH 11.2,	34.6	3.32 $\pm$ 0.15	226 $\pm$ 6	68 $\pm$ 5	11.0 $\pm$ 1.3	86 $\pm$ 20	8 $\pm$ 3
	100% ^2H , 0.6% ^{17}O , 23°C							
Immunoglobulin G	14 mg/ml, pH 10.9,	34.6	2.98 $\pm$ 0.13	210 $\pm$ 6	70 $\pm$ 5	12.3 $\pm$ 1.3	52 $\pm$ 19	4 $\pm$ 2
	100% ^2H , 0.6% ^{17}O , 23°C							
Theory					73.5			230

^aThe ratio varied with pH (cf. Fig. 2)

FIGURE CAPTIONS

Fig. 1. Temperature dependence of the ^{17}O (34.6 MHz) and ^2H (39.1 MHz) excess transverse relaxation rates in aqueous (10% ^2H , 0.5% ^{17}O) solutions of lysozyme (80 mg/ml, pH 3.87 (circles) or 4.14 (squares)) and hemocyanin 1/1 (20 mg/ml, pH 9.03). Arrows indicate re-measurement after exposure to highest temperature. Note the scale shift for the ^2H HC data. The dotted line corresponds to $\ln[\eta(\text{H}_2\text{O})/c\text{P}] + 4.7$ (data from ref. 39).

Fig. 2. Normalized ^{17}O (34.6 MHz) and ^2H (39.1 MHz) excess relaxation rates versus measured pH_D in aqueous (100% ^2H , 0.5% ^{17}O) lysozyme solutions at 23°C . Open symbols refer to R_1 , filled symbols to R_2 . The excess rates were calculated with $R_F(^{17}\text{O}) = 192 \text{ s}^{-1}$ and $R_F(^2\text{H}) = 2.45 \text{ s}^{-1}$. No R_2 data from the pH range where scalar relaxation contributes significantly are included in the figure (cf. experimental section). Squares represent a solution of 82 mg/ml which was titrated with DCl (2% maximum dilution) and circles represent a solution of 84 mg/ml which, after DCl addition to pH_D 2.17, was titrated with NaOD (6% maximum dilution).

Fig. 3. Deuteron longitudinal relaxation dispersion (data from Fig. 1 of ref. 19) in an aqueous (80% ^2H) solution of lysozyme (210 mg/ml) at 4°C . The dotted line corresponds to the relaxation rate in the protein-free, deoxygenated solvent. The solid curve resulted from a least-squares fit of eq. (1a), with $\alpha/R_F = 2.66$, $\beta/R_F = 7.31$ and $\tau_C = 91.5$ ns.

Fig. 4. Deuteron longitudinal relaxation dispersion (data from Fig. 3 of ref. 19) in an aqueous (80% ^2H) solution of hemocyanin 1/1 (30.6 mg/ml) at 25°C . The dotted line corresponds to the relaxation rate in the protein-free, deoxygenated solvent. The curves resulted from least-squares fits of eq. (1a) [dashed curve, parameters: $\alpha/R_F = 1.51$; $\beta/R_F = 13.77$; $\tau_C = 0.757$ μs] and eq. (11) [solid curve, parameters: $\alpha/R_F = 1.44$; $\beta_B/R_F = 2.11$; $\tau_{BS} = 0.151$ μs ; $\beta_P/R_F = 11.73$; $\tau_{PS} = 1.07$ μs]. In both fits, $R_1(0)/R_F$ was fixed to the average ($=15.28$) of the seven data points at frequencies $\nu \leq 10$ kHz.

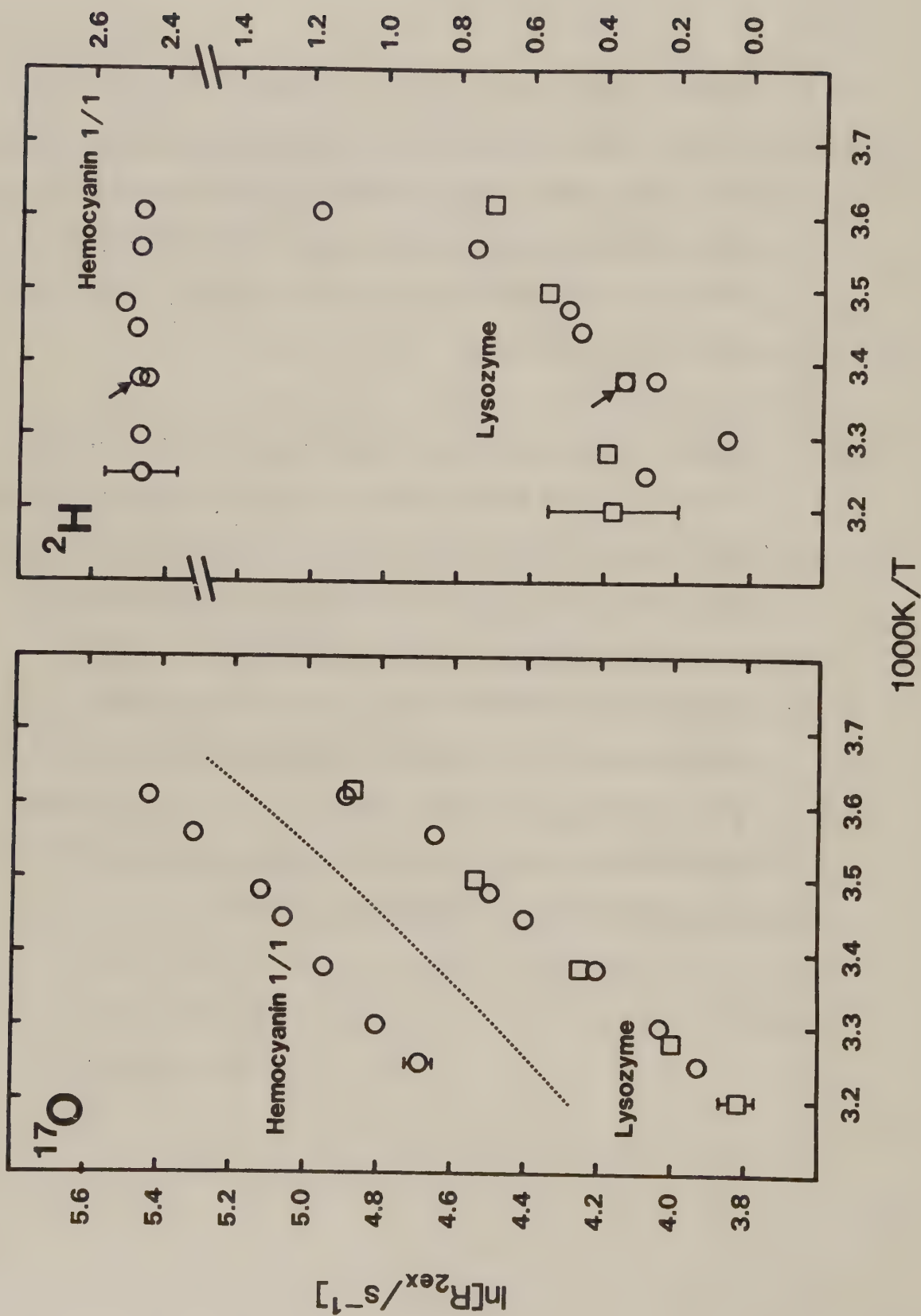
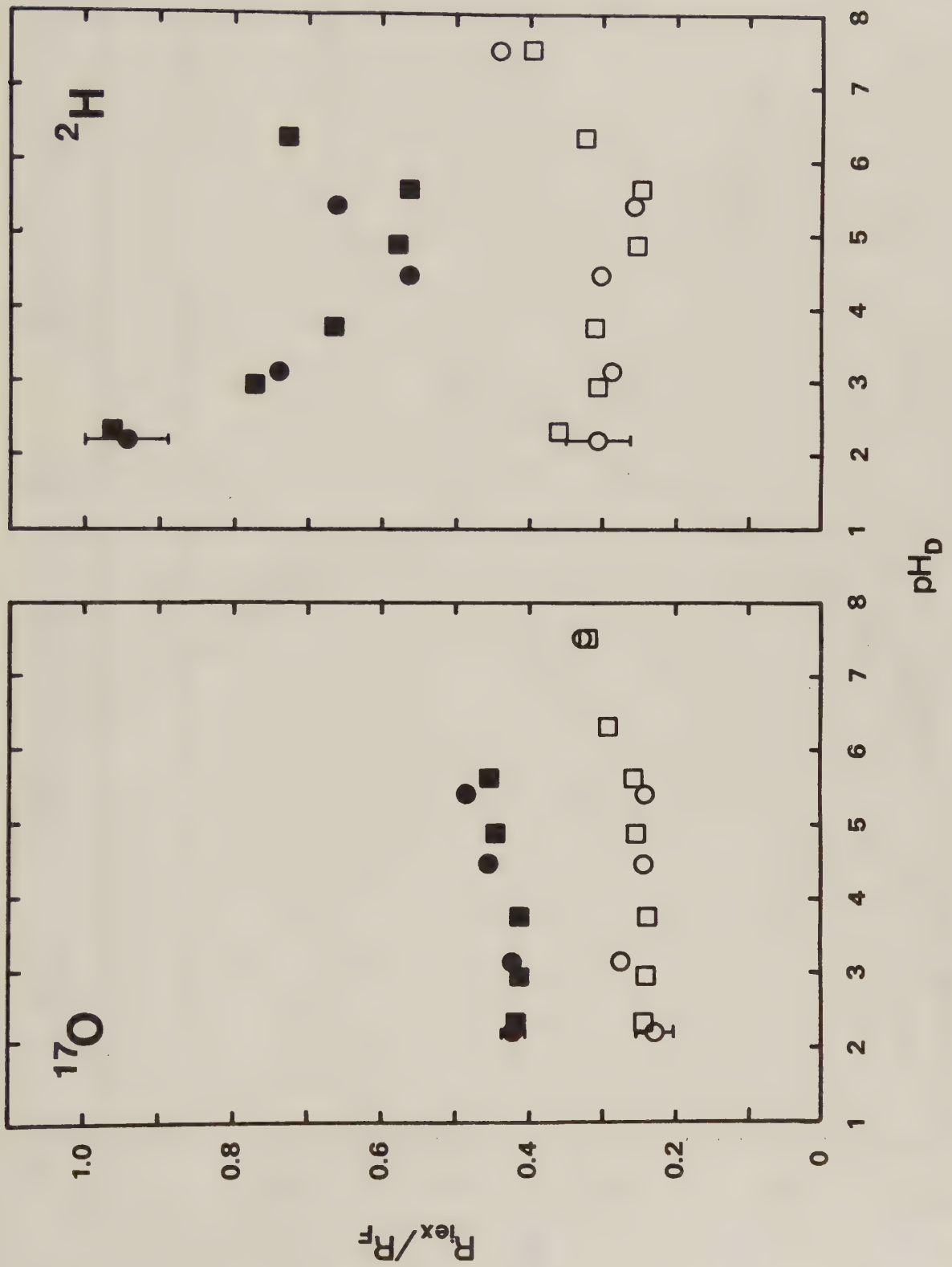


FIGURE 1

FIGURE 2



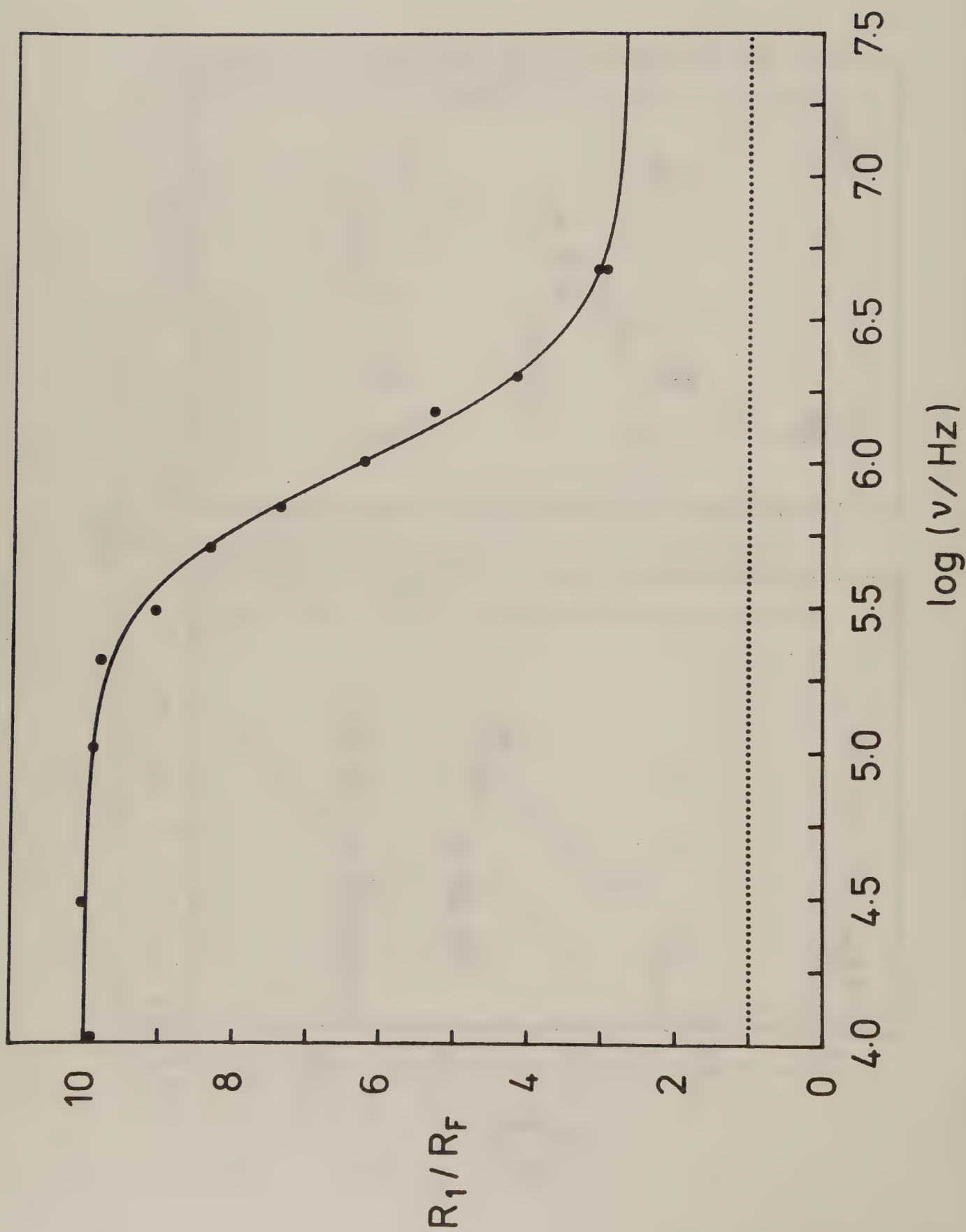


FIGURE 3

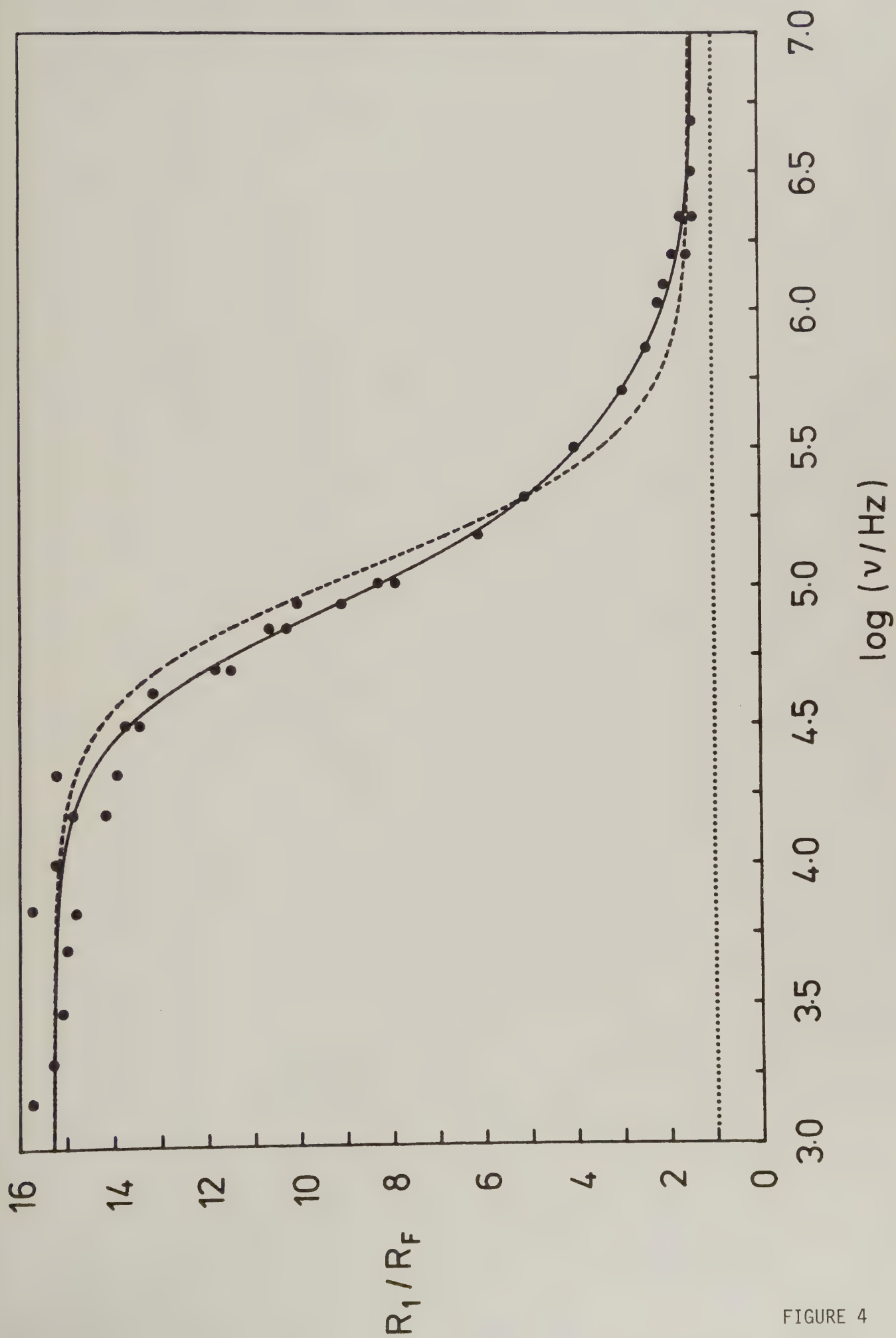


FIGURE 4

VII

WATER SPIN RELAXATION IN COLLOIDAL SYSTEMS

Part 3. Interpretation of the Low-Frequency Dispersion

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Running title: Interpretation of Water NMR in Colloidal Systems

ABSTRACT

The contribution to the water ^{17}O spin relaxation from slow molecular processes in colloidal solutions has been interpreted in terms of a general theory of spin relaxation in locally ordered fluids, coupled with a dynamic model which describes the translational motion of water molecules by a diffusion equation with an anisotropic and position-dependent diffusivity tensor. This model can explain all the experimental observations provided that the lateral and radial diffusivities of water molecules within ca 1 nm of the colloid surface are reduced by 1-2 and 2-3 orders of magnitude, respectively, compared to bulk water. The dynamic coupling between water motion and rotational diffusion of colloidal particles does not affect water ^{17}O spin relaxation for particle radii in excess of ca 10 nm.

1. INTRODUCTION

It is by now a well-established experimental fact that the spin relaxation behaviour of water nuclei in colloidal solutions differs qualitatively from that in solutions of low molecular weight solutes¹⁻⁸. Since this qualitative difference is manifest in ^{17}O relaxation⁵⁻⁸, it necessarily reflects the interactions and dynamics of water molecules. The fundamental problem is to understand how the large size of the colloidal solute can give rise to a long-time tail in the orientational time correlation function and hence to a low-frequency relaxation dispersion and a concomitant inequality of the longitudinal and transverse relaxation rates. The present paper - the last in a series of three - is devoted primarily to this problem.

Our point of departure will be a recently developed formalism for spin relaxation in locally ordered fluids⁹⁻¹¹. This treatment is quite general and the relevant molecular degrees of freedom are described in terms of equilibrium and time-dependent distribution functions. A dynamic model is then specified, according to which the translational motion of a water molecule, relative to a colloidal particle, is governed by a continuous diffusion equation with an anisotropic and position-dependent diffusivity tensor. The diffusivity tensor accounts, in a phenomenological way, for dynamic many-body correlations, including a dynamic coupling to the reorientational motion of the colloidal particle. Previous interpretations of water spin relaxation data from colloidal solutions have usually involved parameters, such as the "bound water lifetime", which are opera-

tional to some extent. In contrast, the diffusivity tensor of the present dynamic model is a well-defined non-operational quantity, allowing contact to be made with other types of experiment, with computer simulation studies, and with kinetic theory.

Part 1 in this series of papers⁷ reports extensive water ^{17}O relaxation data from colloidal silica dispersions. This simple model system has the important advantage that the radius of the spherical silica particles can be varied while the surface morphology remains the same. We find that the diffusion model is consistent with all the experimental observations on this system, provided that the translational diffusivity within the first few water layers adjacent to a colloidal particle is reduced from the bulk value by several orders of magnitude. This result has implications for many kinetic processes taking place at interfaces in aqueous systems. Water ^{17}O relaxation data from protein solutions, reported in ref. 5 and in Part 2 of this series of papers⁸, while less straightforward to interpret in detail, nevertheless do conform to the general picture of water dynamics in colloidal solutions emerging from this study.

2. THEORY

Much of the material in this section is developed at greater length in ref. 9. In the following we present only some key results, the emphasis being on the underlying physical concepts rather than on the mathematical details.

2.1. SPIN RELAXATION IN LOCALLY ORDERED FLUIDS

In the conventional theory of nuclear spin relaxation the molecular degrees of freedom enter by way of an orientational time correlation function, determined by the spectrum of orientational fluctuations of - in the present case - a water molecule with respect to an external lab-fixed coordinate frame. The anisotropic interactions experienced by water molecules in the vicinity of the colloid surface have two effects: they perturb the reorientational motion of nearby water molecules and - more importantly - they give rise to a local orientational ordering, or polarization, of the adjacent fluid. As a result of the local ordering the correlation function does not decay completely on the timescale of the local (perturbed) water reorientation, but acquires a long-time tail associated with those processes that randomize the orientation of the local polarization axis with respect to the lab-fixed frame. Since, in a colloidal solution, these latter processes are much slower than the local reorientation, the total correlation function may be decomposed as⁹⁻¹¹

$$G(t) = G_f(t) + G_s(t) \quad (1)$$

Henceforth we shall focus on the part, $G_s(t)$, associated with the slower processes.

Since the orientational ordering, as probed in NMR experiments, extends only a few water molecules away from the surface¹¹⁻¹³, it is reasonable

to model it as a step-function perturbation. Accordingly, we recognize a polarized region extending a distance δ away from the colloid surface and an unpolarized region encompassing the remaining water molecules. For historic reasons we denote these regions, or states, by B and F, respectively. The correlation function $G_S(t)$ now involves two stochastic variables: the angle, $\theta(t)$, between the local polarization axis and the external static magnetic field, and the state variable⁹, $\Gamma(t)$, which indicates whether a water molecule is in state B or in state F. Given these premises, one may express the correlation function as⁹

$$G_S(t) = P_B \bar{V}_B^2 \int_{-1}^1 d\xi_0 f(\xi_0) P_2(\xi_0) \int_{-1}^1 d\xi f(\xi, B; t | \xi_0, B) P_2(\xi) \quad (2)$$

where $P_2(\xi)$ is the second-degree Legendre polynomial, $\xi \equiv \cos \theta$, P_B is the fraction of water molecules in the B state and $\bar{V}_B$ is the locally averaged residual quadrupole (or spin-lattice) coupling⁹ in the B state. Furthermore, $f(\xi, B; t | \xi_0, B) d\xi$ is the probability that a water molecule, initially in state B with local polarization axis orientation ξ_0 , at time t resides in state B with a polarization orientation to within $d\xi$ of ξ . In an isotropic colloidal solution, the orientational equilibrium distribution is $f(\xi) = \frac{1}{2}$.

We emphasize that eq. (1) and (2) are only very weakly model-dependent; they rely only on general symmetry and timescale arguments and on the replacement of the short-ranged polarization profile by a step-function. The residual coupling, $\bar{V}_B$, can be evaluated for specific interaction models and the joint propagator, $f(\xi, B; t | \xi_0, B)$ can be obtained by solving an appropriate stochastic equation of motion. To this latter problem we now turn our attention.

2.2. THE CONTINUOUS DIFFUSION MODEL

To proceed beyond eq. (2) we must identify the dynamic processes that give rise to fluctuations in the stochastic variables ξ and Γ appearing in the propagator $f(\xi, \Gamma; t | \xi_0, \Gamma_0)$. We consider first a hypothetical system where the colloidal particles are prevented from rotating. In such a system the propagator can only be affected by the translational motion of water molecules; radial translations modulate the state variable Γ , while lateral translations modulate the orientation variable ξ . We assume that the translational motion of a water molecule relative to a colloidal particle can be described by a diffusion equation in the radial (r) and orientational (ξ) coordinates;

$$\frac{\partial}{\partial t} f(r, \xi; t | r_0, \xi_0) = \underline{\nabla} \cdot \underline{D}(r) \cdot \underline{\nabla} f(r, \xi; t | r_0, \xi_0) \quad (3)$$

To solve eq. (3) we need two boundary conditions. Since a water molecule cannot penetrate the colloidal particle, we impose a reflection boundary condition at the colloid surface;

$$\frac{\partial}{\partial r} f(r, \xi; t | r_0, \xi_0) \big|_{r=a} = 0 \quad (4a)$$

where a denotes the radius of the colloidal particle. The second boundary condition is obtained by introducing the dynamic cell approximation^{9,10} (DCA). In the DCA the system is represented by a collection of spherical cells of radius b , each with a colloidal particle at its center, and the

orientational variable ξ is taken to be completely randomized by the time a water molecule, initially in state B, reaches the cell boundary at $r=b$. The subsequent fate of the water molecule is then irrelevant so that we may impose an absorption boundary condition at the cell boundary;

$$f(b, \xi; t | r_0, \xi_0) = 0 \quad (4b)$$

If diffusional reencounters with the B state are unimportant - and we shall see that they are - then the nature of the boundary condition at $r=b$ has no effect on $G_s(t)$ and the DCA is no longer needed.

The propagator in eq. (2) is obtained from that in eq. (3) by integrating over r within state B and averaging over r_0 within state B;

$$f(\xi, B; t | \xi_0, B) = \frac{1}{P_B} \int_a^{a+\delta} dr_0 r_0^2 f(r_0) \int_a^{a+\delta} dr r^2 f(r, \xi; t | r_0, \xi_0) \quad (5)$$

The radial equilibrium distribution is given by

$$f(r) = \frac{3}{(b^3 - a^3)} \quad (6)$$

and the cell radius, b , is related to the colloid number density, n_c , through

$$b = \left(\frac{3}{4\pi n_c} \right)^{1/3} \quad (7)$$

Because of the spherical symmetry of the system, the diffusivity tensor appearing in eq. (3) may be expressed as

$$\underline{\underline{D}}(r) = D_{\text{rad}}(r) \underline{\hat{r}} \underline{\hat{r}} + D_{\text{lat}}(r) [\underline{\underline{I}} - \underline{\hat{r}} \underline{\hat{r}}] \quad (8)$$

where $\underline{\underline{I}}$ is the unit dyadic and $\underline{\hat{r}}$ is the radial unit vector. The r -dependence of the radial and lateral water self-diffusion coefficients accounts for perturbations of the translational motion induced by the nearby stationary colloid surface. Far away from the surface both D_{rad} and D_{lat} attain the bulk value, D_0 , but near the surface they are expected to be smaller than D_0 .

Until now we have regarded the colloidal particles as stationary, whereas, in fact, they undergo rotational diffusion. How does this motion affect the correlation function $G_s(t)$? Because of their incessant "collisions" with the colloidal particle, the adjacent water molecules acquire a zero average lateral velocity component relative to the moving colloid surface. In hydrodynamics this phenomenon is described by a so-called stick boundary condition for the fluid velocity field at the surface. This does not, of course, imply that water molecules are translationally immobile at the surface; a stick boundary condition is fully compatible with a diffusional spread (characterized by D_{lat}) at the surface. Because of the dynamical coupling, as described hydrodynamically by the stick boundary condition, the colloid rotation affects the water translation relative to a lab-fixed frame by superimposing a lateral drift on the lateral spread. (Remember that the orientational variable ξ is defined with respect to a

lab-fixed frame.) The effectiveness of the dynamical coupling decreases with the distance from the colloid surface as reflected, in the hydrodynamic limit, by the radial decay of the velocity field¹⁴.

Since the surface-induced perturbation of the radial and lateral water diffusion is expected to be short-ranged, we shall assume a step-function perturbation with constant D_{rad} and D_{lat} within the orientationally ordered B state and with $D_{\text{rad}} = D_{\text{lat}} = D_0$ in the F state. If the same assumption is made for the dynamic coupling we have

$$\underline{D}(r) = \begin{cases} D_{\text{rad}} \underline{\hat{r}} \underline{\hat{r}} + D'_{\text{lat}} (\underline{I} - \underline{\hat{r}} \underline{\hat{r}}) & , \quad a < r < a + \delta \\ D_0 \underline{I} & , \quad a + \delta < r < b \end{cases} \quad (9)$$

with an effective lateral diffusion coefficient

$$D'_{\text{lat}} \equiv D_{\text{lat}} + a^2 D_{\text{rot}} = D_{\text{lat}} + \frac{kT}{8\pi\eta a} \quad (10)$$

where the Stokes-Einstein relation was used to relate the colloid rotational diffusion coefficient to its radius and to the solvent viscosity.* Unless $\delta \ll a$, the colloid radius, a , in eq. (10) should be replaced by an effective radius for state B.

* The hydrodynamic interaction between the colloidal particles leads to a reduction of D_{rot} by a factor of $1 - \frac{5}{16} \left(\frac{a}{b}\right)^3$, assuming a pairwise additive hydrodynamic interaction¹⁵. For the data to be considered here, not only is this correction small (<7%) but $G_s(t)$ is virtually independent of D_{rot} . We can thus safely use eq. (10).

If diffusional reencounters with the B state are unimportant, as seems to be the case (see below), then the dynamics in state F has no effect on $G_S(t)$. The fact that the dynamic coupling between water translation and colloid rotation extends into the F state is then irrelevant.

In principle, the dynamic coupling also affects $G_F(t)$ in eq. (1) by superimposing on the fast water reorientation a much slower motion which also modulates the orientation of a water molecule with respect to a lab-fixed frame. This will hasten the decay of $G_F(t)$ and hence reduce the relaxation rate. This effect, which because of the vast timescale separation is utterly negligible, is the only consequence of the dynamic coupling in the absence of orientational ordering at the colloid surface.

The dynamic model is now complete and we may solve the diffusion equation (3), with the boundary conditions (4) and the diffusivity tensor (9), for the propagator $f(r, \xi; t | r_0, \xi_0)$, which, in combination with eq. (2), (5) and (6), yields the correlation function $G_S(t)$. However, since we shall be interested mainly in the contribution of $G_S(t)$ to the spin relaxation rate at zero frequency, it suffices to solve for the time integral of $G_S(t)$, which may be obtained in closed form⁹. The slow-motion contribution to the zero-frequency relaxation rate is given by¹⁶

$$R_S(0) = \frac{(2I + 3)}{(2I)^2(2I - 1)} \left(\frac{eQ}{\hbar} \right)^2 \int_0^\infty dt G_S(t) \quad (11)$$

where I is the nuclear spin quantum number and eQ is the nuclear quadrupole moment. From eq. (2) - (11) and the relation⁹⁻¹¹ $eQ\bar{V}_B/h = \left(\frac{3}{2}\right)^{\frac{1}{2}} A_X$,

where A is the residual anisotropy¹¹ and χ is the quadrupole coupling constant, one obtains without further approximations⁹

$$R_s(0) = \frac{9\pi^2}{20} \frac{(2I+3)}{I^2(2I-1)} (A\chi)^2 \phi \frac{a^2}{D_{\text{rad}}} [s(\alpha\eta^{2s} - \beta)]^{-1} \quad (12a)$$

$$\times \left[\left(\frac{\eta^{s_+} - 1}{s_+} \right)^2 + \alpha\beta\eta^5 \left(\frac{\eta^{s_-} - 1}{s_-} \right)^2 + \frac{2(\alpha+\beta)}{s_+s_-} \eta^{s_+} + \frac{2}{5}\eta^5 \left(\frac{\alpha}{s_+} \eta^{2s_-} - \frac{\beta}{s_-} \right) - \frac{2}{5} \left(\frac{\alpha}{s_-} \eta^{2s_+} - \frac{\beta}{s_+} \right) \right]$$

where we have defined

$$\alpha \equiv \frac{(s-\frac{1}{2})(1-\lambda^5\eta^5)D_{\text{rad}} + (3+2\lambda^5\eta^5)D_0}{(s+\frac{1}{2})(1-\lambda^5\eta^5)D_{\text{rad}} - (3+2\lambda^5\eta^5)D_0} \quad (12b)$$

$$\beta \equiv \frac{(s-\frac{1}{2})}{(s+\frac{1}{2})} \quad (12c)$$

$$s_{\pm} \equiv s \pm \frac{5}{2} \quad (12d)$$

$$s \equiv \left(6 \frac{D'_{\text{lat}}}{D_{\text{rad}}} + \frac{1}{4} \right)^{\frac{1}{2}} \quad (12e)$$

$$\phi \equiv \frac{\lambda^3}{(1 - \lambda^3)} \quad (12f)$$

$$\lambda \equiv \frac{a}{b} \quad (12g)$$

$$\eta \equiv \frac{(a + \delta)}{a} \quad (12h)$$

Equation (12) is valid only for $D'_{lat} \neq D_{rad}$; the result for $D'_{lat} = D_{rad}$ is given elsewhere⁹ but will not be needed here. With b and D'_{lat} given by eq. (7) and (10), respectively, eq. (12) contains four unknown parameters: A , δ , D_{rad} and D_{lat} .

2.3. NEGLECT OF REENCOUNTERS

From a physical point of view it is clear that as the radial diffusion in state B becomes much slower than in state F diffusional reencounters with state B will no longer affect $R_s(0)$. This follows because $\int_0^\infty dt G_s(t)$ is essentially a measure of the accumulated time that a water molecule has spent in state B before the local polarization direction has become randomized, and if $D_{rad} \ll D_0$, then virtually all of that time will be associated with the original visit in state B. Mathematically, diffusional reencounters with state B can be eliminated by imposing an absorption boundary condition at $r=a+\delta$, rather than at $r=b$ as in eq. (4b). It can be shown⁹ that this leads to eq. (12) with $\alpha=-1$, corresponding to the limit $D_{rad}/D_0 \rightarrow 0$. With $\alpha=-1$, eq. (12) shows that $R_s(0)$ is proportional to the colloid-to-water volume ratio, ϕ , with no other dependence on the concentration of colloidal particles. This is expected, for if all the relevant dynamics takes place within state B, then the polarized region of each colloidal particle contributes independently to $R_s(0)$.

When diffusional reencounters with state B may be neglected, the predictions of the continuous diffusion model (CDM), as presented in section 2.2., agree with those of the discrete exchange model (DEM), in which the radial motion is modelled as an uncorrelated random walk among discrete states⁹. According to the DEM¹¹

$$R_s(0) = \frac{3\pi^2}{10} \frac{(2I+3)}{I^2(2I-1)} (A_X)^2 P_B \tau_{Bs} \quad (13)$$

where τ_{Bs} is an effective correlation time characterizing dynamic processes within the B state.

As in the CDM, we consider three independent processes: lateral water diffusion, dynamic coupling to colloid rotational diffusion and radial water diffusion. By itself, each of the first two processes would lead to a strictly exponential correlation function, with correlation times τ_{lat} and τ_{rot} , respectively. The correlation function corresponding to radial water displacements is also exponential in the DEM, with a correlation time, τ_{res} , which is the mean residence time of a water molecule in state B. The effective correlation time may now be written as

$$\frac{1}{\tau_{Bs}} = \frac{1}{\tau_{lat}} + \frac{1}{\tau_{rot}} + \frac{1}{\tau_{res}} \quad (14)$$

with

$$\tau_{lat} = \frac{a^2}{6D_{lat}} \quad (15)$$

$$\tau_{rot} = \frac{1}{6D_{rot}} \quad (16)$$

To make contact with the CDM result, eq. (12), we evaluate τ_{res} for a diffusion model (within state B), which yields⁹

$$\tau_{\text{res}} = \frac{\delta^2}{3D_{\text{rad}}} \quad (17)$$

On combining eq. (13)-(17) we obtain, for $\delta \ll a$,

$$R_s(0) = \frac{3\pi^2}{10} \frac{(2I+3)}{I^2(2I-1)} (A_X)^2 \phi \frac{a^2}{D_{\text{rad}}} \left(\frac{\delta}{a}\right)^3 \left[1 + 2\left(\frac{\delta}{a}\right)^2 \frac{D'_{\text{lat}}}{D_{\text{rad}}} \right]^{-1} \quad (18)$$

where we have expanded P_B to first order in δ/a : $P_B = (\eta^3 - 1)\phi \approx 3\delta\phi/a$. For $\delta/a < 0.1$ and $D'_{\text{lat}}/D_{\text{rad}} < 100$, eq. (12a) with $\alpha = -1$ and eq. (18) agree to within 1%. Eq. (18) has the advantages of a simpler structure and of involving only three independent unknowns, e.g. δA^2 , δ^2/D_{rad} and D_{lat} . For sufficiently large colloidal particles, with $a^2 D_{\text{rot}} \ll D_{\text{lat}}$, the number of independent parameters is further reduced by one; the two composite parameters may be chosen as $\delta^3 A^2/D_{\text{rad}}$ and $\delta^2 D_{\text{lat}}/D_{\text{rad}}$.

3. COMPARISON WITH EXPERIMENT

3.1. GENERAL CONSIDERATIONS

In Part 1 we reported ¹⁷O longitudinal and transverse relaxation rates from dispersions of colloidal silica particles of variable size and concentration and at several resonance frequencies and temperatures. This extensive set of data will now be used to test the

dynamic model described in section 2. Some reference will be made also to ^{17}O relaxation data from protein solutions^{5,8}.

To be acceptable, the dynamic model must account for the following experimental observations from the ^{17}O study⁷ of colloidal silica particles:

- (1) a low-frequency relaxation dispersion corresponding to molecular motion on a timescale of $\gtrsim 10^{-8}\text{s}$,
- (2) a large difference between R_1 and R_2 in the plateau region of the dispersion, corresponding to a large $R_S(0)$,
- (3) a proportionality of $R_S(0)$ and the colloid-to-water volume ratio throughout the investigated range $0 < \phi < 0.3$,
- (4) a reduction of $R_S(0)/\phi$ with increasing particle size in the range $10 < a < 40\text{ nm}$,
- (5) an apparent activation energy for $R_S(0)$ which does not exceed that for relaxation in pure water.

At the outset we must establish a connection between the observed longitudinal and transverse relaxation rates and the contribution, $R_S(0)$, from slow processes to the zero-frequency relaxation rate. We shall be concerned mainly with data from the plateau region of the relaxation dispersion, where the slow processes contribute only to the transverse relaxation so that $R_S(0)$ is proportional to $R_2 - R_1$. Since the ^{17}O relaxation is in the "nearly exponential" regime¹⁷ (cf section 2.3. in Part 1), the

proportionality factor is 10/3 and

$$R_s(0) = \frac{10}{3} (R_2 - R_1) \quad (19)$$

(This relation actually remains numerically accurate in the high-frequency part of the dispersion region.)

As concentration variable we shall use the colloid-to-water volume ratio, ϕ , defined by eq. (7), (12f) and (12g). To convert from the corresponding mass ratios given in Part 1, we used a ratio of silica to water densities of 2.2. In all calculations we shall use particle radii deduced from dynamic light scattering measurements (cf Table 1 in Part 1). For given particle size and concentration, eq. (12) shows that $R_s(0)$ depends on the four parameters A , δ , D_{rad} and D_{lat} . Fortunately, independent information is available about A and δ , which characterize the extent and range of orientational order at the colloid surface. From studies of quadrupolar line splittings in the water NMR spectra from clays¹² and lyotropic liquid crystals¹³, it has been concluded that the polarized region extends one or two water "layers" into the fluid. We shall therefore regard $\delta=1$ nm as an upper limit in our calculations. Furthermore, we shall use a typical* value of $A=0.05$ throughout; the sensitivity of our conclusions to this choice is indicated below. For the ^{17}O quadrupole coupling constant we will use the librationaly averaged¹⁸ value $\chi=6.67$ MHz¹¹.

* In Part 1, we deduce $0.02 \leq |A| \leq 0.06$ from water relaxation rates in colloidal silica. Information about this parameter can also be obtained from quadrupolar line splittings in ordered systems such as clays. The silicon oxide surface of expandable montmorillonite clays, having a charge density²⁵ of rough

3.2. UNPERTURBED WATER DIFFUSION

Let us first consider the case of unperturbed water diffusion. With $D_{\text{rad}}=D_{\text{lat}}=D_0$ and a plausible upper limit of $\delta=1$ nm, eq. (12) yields $R_s(0)$ values smaller than the experimental ones by an order of magnitude. As shown in Fig. 1, agreement with experiment at low volume ratios requires much larger δ values (2.7-3.8 nm, for $A=0.05$, depending on particle size), which are difficult to reconcile with previous conclusions about the range of the surface-induced polarization^{12,13}.

There are two further arguments against a model with unperturbed water diffusion. Firstly, as seen from Fig. 1 it is not possible to reproduce the observed linear ϕ -dependence with $D_{\text{rad}}=D_{\text{lat}}=D_0$. The precise form of the ϕ -dependence is influenced by the DCA (absorbing cell boundary), but it seems unlikely that an exactly linear relationship would emerge, for both particle sizes, if one were to relax the DCA. Secondly, as will be shown in section 3.5., the observed frequency dependence of R_s deviates from that predicted theoretically with $D_{\text{rad}}=D_{\text{lat}}=D_0$.

one negative charge per 1.4 nm^2 , resembles that of the silica particles referred to here⁷. With $|A(^{17}\text{O})/A(^2\text{H})| = 2$ and $\chi(^2\text{H}) = 222 \text{ kHz}$ (ref. 11), the ^2H quadrupolar line splittings measured by Woessner¹² in expandable clays of varying water layer thickness (2.3-25 nm) and with varying counterion (Li, Na, K, Rb, Ca) yield values in the range 0.035-0.079 for the composite quantity $|A(^{17}\text{O})|\delta/\text{nm}$.

3.3 RADIAL WATER DIFFUSION

Having rejected models with unperturbed water diffusion, we now examine the effect of reducing the diffusivity near the colloid surface. It follows from eq. (12) that the effect of preventing lateral diffusion in state B ($D_{lat}=0$) without perturbing the radial diffusion ($D_{rad}=D_0$) is to increase $R_S(0)$ by a mere 10-20% compared to the case of unperturbed diffusion. This insensitivity to the lateral diffusivity in state B comes about because, as long as $\delta \ll a$ and $D_{rad} \approx D_0$, the local polarization direction can be randomized by lateral diffusion in state F in just slightly longer time. In contrast, $R_S(0)$ is very sensitive to the radial diffusivity in state B. Fig. 1 shows that with D_{rad} reduced from D_0 by a factor of 200-500, we can reproduce not only the observed magnitude of $R_S(0)$, using a reasonable $\delta=1$ nm, but also the linear ϕ -dependence. As will be shown in section 3.5., also the observed frequency dependence of R_S can now be explained.

With $D_{rad} \ll D_0$, eq. (12b) yields $\alpha \approx -1$, which, as we have already remarked, corresponds to neglect of diffusional reencounters with state B. The DEM results of section 2.3. are then sufficiently accurate for the dynamics to be described quantitatively in terms of the three correlation times τ_{lat} , τ_{rot} and τ_{res} .

3.4. LATERAL WATER DIFFUSION

In the DEM regime, i.e. with $D_{\text{rad}} \ll D_0$, eq. (14) shows that lateral water diffusion will affect $R_s(0)$ unless τ_{lat} is much longer than either τ_{res} or τ_{rot} . For the largest colloidal particle investigated ($a=40$ nm) τ_{lat} exceeds τ_{res} even with $D_{\text{lat}}=D_0$. Agreement with the experimental $R_s(0)$ versus ϕ data is therefore obtained for any $D_{\text{lat}} \leq D_0$ without requiring D_{rad} to vary by more than a factor of 2 (see Fig. 1b). However, for the smaller particle size of Fig. 1a ($a=12$ nm) τ_{lat} can become shorter than τ_{res} and τ_{rot} and thus influence $R_s(0)$. In fact, with $\delta=1$ nm and $A=0.05$, the theoretical $R_s(0)$ values fall short of the experimental ones for $D_{\text{lat}}/D_0 > 0.4$, irrespective of the value of D_{rad} . By examining how $R_s(0)$ varies with particle size it should therefore be possible to determine the lateral diffusivity.

The dependence of $R_s(0)$ on the radius of the colloidal particles at two temperatures is shown in Fig. 2. Since the data were obtained at different colloid concentrations we have plotted the quantity $R_s(0)/\phi$, which, according to Fig. 1, is independent of concentration. The theoretical prediction in the case that $D_{\text{rad}} \ll D_0$ is that $R_s(0)/\phi$ will increase with the particle radius, a , for small particles (τ_{lat} or $\tau_{\text{rot}} < \tau_{\text{res}}$), go through a maximum and then, for sufficiently large radii ($\tau_{\text{res}} < \tau_{\text{lat}}$ and τ_{rot}), decrease with a . Clearly, the investigated particle sizes fall in the latter category. In accordance with the discussion of Fig. 1, $R_s(0)$ is essentially independent of D_{lat} for the largest particles, whereas, for the smallest particles, it depends strongly on D_{lat} ; as D_{lat} decreases

the peak in $R_s(0)/\phi$ becomes larger and shifts to smaller radii. The fits to eq. (12) in Fig. 2 show that the lateral diffusivity in state B is reduced by an order of magnitude; at 27°C we obtain $D_{\text{rad}}/D_0=0.003$ and $D_{\text{lat}}/D_0=0.1$ (with $\delta=1$ nm). The shaded regions in Fig. 2 are bounded by curves calculated with $D_{\text{rot}}=0$ and with D_{rot} as given by eq. (10). Clearly, the effect of dynamic coupling to colloid rotation is insignificant for particles with radius exceeding about 10 nm.

As expected, the two- and three-parameter versions of the DEM eq. (18) also reproduce the observed size dependence accurately. The two-parameter fit at 27°C resulted in $\delta^3(A_X)^2/D_{\text{rad}}=1.7\times 10^{-5} \text{ ms}^{-1}$ and $\delta^2 D_{\text{lat}}/D_{\text{rad}}=4.4\times 10^{-17} \text{ m}^2$. With $A_X=3.4\times 10^5 \text{ s}^{-1}$, $\delta=1$ nm and $D_0=2.4\times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ this yields $D_{\text{rad}}/D_0=0.003$ and $D_{\text{lat}}/D_0=0.1$. The sensitivity of the deduced diffusivities to the assumed values of A_X and δ can be estimated from the composite parameters: $D_{\text{rad}} \sim \delta^3(A_X)^2$, $D_{\text{lat}} \sim \delta(A_X)^2$ and $D_{\text{rad}}/D_{\text{lat}} \sim \delta^2$. These dependences may appear discouraging, but again we emphasize that a large bulk of experimental data impose rather narrow limits on δ and A_X .

Another source of uncertainty in the determined radial and lateral diffusivities is the imperfect characterization of the silica particles. For the larger particles, dynamic light scattering measurements gave radii twice as large as those determined from measurements of surface area and the discrepancy is even larger for the smallest particles (see Table 1 in Part 1). This difference may be due partly to surface roughness, which increases the surface area and hence leads to too small radii from surface area determinations. In our model, surface roughness would affect the parameters δ (increase) and A (decrease in magnitude). Furthermore, sample

polydispersity, which is pronounced for the smallest particles (see Table 1 in Part 1), may contribute to the divergent results of the two methods. Despite these uncertainties, we regard the deduced order of magnitude of the radial and lateral diffusivities as reliable. Neglecting particle rotation (cf Fig. 2), the particle radius and the diffusivities enter eq. (18) in the combinations $D_{\text{rad}}a$ and D_{lat}/a . A reduction of all radii with a factor of 2 thus corresponds to D_{rad} being twice as large and D_{lat} twice as small. Indeed, with particle radii from surface area measurements (see Table 1 in Part 1) and diffusivities differing by a factor of 2 from those of Fig. 2, eq. (12) yields equally good fits to the data as those based on light scattering radii.

3.5. RELAXATION DISPERSION

Fig. 3 shows the frequency dependence of the slow-motion contribution, R_{1s} , to the longitudinal ^{17}O relaxation rate for silica particles of radius 12.1 nm at $\phi=0.113$. The experimental data were obtained from Table 2 in Part 1, using $R_f=171 \text{ s}^{-1}$ and $R_s(0)=118 \text{ s}^{-1}$.

For the case $D_{\text{rad}}=D'_{\text{lat}}=D_0$, the diffusion equation (3) can be solved directly in terms of spherical Bessel functions⁹, thereby yielding $G_s(t)$ and $R_{1s}(\nu_0)$. The dashed curve in Fig. 3 is the resulting relaxation dispersion with $\delta=3.8 \text{ nm}$, as required for agreement with experiment at low concentrations in Fig. 1a ($\delta=1 \text{ nm}$ yields a dispersion which is shifted slightly towards higher frequencies). We conclude that a dynamic model with unperturbed water diffusion predicts a too high dispersion frequency.

In the DEM regime, the dispersion of R_{1s} is given by

$$\frac{R_{1s}(\nu_0)}{R_s(0)} = \frac{0.2}{1+(2\pi\nu_0\tau_{Bs})^2} + \frac{0.8}{1+4(2\pi\nu_0\tau_{Bs})^2} \quad (20)$$

where τ_{Bs} is the effective correlation time of eq. (14). With the parameters obtained from curve 1 in Fig. 2, eq. (14)-(18) yield $\tau_{Bs}=32.5$ ns for a particle radius of 12.1 nm. With this correlation time eq. (20) produces the solid curve in Fig. 3, in satisfactory agreement with experiment.

3.6. THE FAST EXCHANGE CONDITION

Having established the approximate validity of the DEM, and thus also of the concept of local relaxation rates in each region or state, we may ask whether the fitted parameters are consistent with fast exchange conditions. With $P_B \ll 1$, the criterion for fast exchange is¹⁹

$$\tau_{res} \ll R_{2B}^{-1} \quad (21)$$

The local relaxation rate in state B is composed of contributions from fast and slow motions and, in analogy with eq. (1),

$$R_{2B} = R_{Bf} + R_{2Bs} \quad (22)$$

To examine the most severe case, we use the maximal rates $R_{Bf} \approx 10R_F$ (cf. Part 1) and $R_{2Bs} \approx \frac{12\pi^2}{125} (A_X)^2 \tau_{res}$. With $R_F = 140 \text{ s}^{-1}$, $A_X = 3.4 \times 10^5 \text{ s}^{-1}$ and $\tau_{res} = 50 \text{ ns}$ at 27°C , we obtain $R_{2B}^{-1} \approx 10^{-4} \text{ s}$;

well in excess of τ_{res} . The fast exchange condition, eq. (21), is thus satisfied and the fitted parameters are consistent with the positive slopes (indicative of fast exchange) in the Arrhenius plots of $\ln R_{2\text{ex}}$ versus $1/T$ in Fig. 1 of Part 1.

3.7. THE ACTIVATION ENERGY

For the smaller silica particles⁷ and for various globular proteins^{5,8} one obtains from the temperature dependence of the ^{17}O relaxation rates Arrhenius activation energies for $R_s(0)$ of $20 \pm 5 \text{ kJ mol}^{-1}$ in the range $0\text{--}30^\circ\text{C}$. On the basis of the weak temperature dependence of water ^2H quadrupole splittings in clays²⁰, we may neglect the temperature dependence of A and δ and ascribe the observed activation energies to the effective correlation time τ_{BS} . If the dynamic perturbation of the water diffusivity, as measured by the ratios D_{rad}/D_0 and D_{lat}/D_0 , is temperature independent - and this is the case in the hydrodynamic limit²¹ - then $R_s(0)$ should have the same activation energy as the bulk water diffusivity D_0 , which is 19 kJ mol^{-1} in this temperature range²². This is the case for the smaller particles, whereas the somewhat lower value for the larger silica particles ($13 \pm 5 \text{ kJ mol}^{-1}$ for $a=41 \text{ nm}^7$) might indicate that D_{rad} has a lower activation energy than D_0 .

So far we have ascribed the long residence time, τ_{res} , in state B to a radial diffusivity, D_{rad} , reduced by dynamical many-body correlations in the neighbourhood of the colloid surface. An alternative interpretation

of τ_{res} would be in terms of an effective potential barrier located at the dividing surface between the B and F states. A water molecule is then regarded as diffusing in a potential of mean force, $w(r) = -kT \ln[n(r)/\bar{n}]$, where $n(r)$ is the local water concentration (or colloid-water distribution function). From computer simulation studies of model fluids in contact with an idealized surface it is known that $n(r)$ oscillates near the surface²³⁻²⁷. Such a barrier, or series of barriers, which is a result of static many-body correlations (packing constraints in the simplest model), if sufficiently high, would prevent diffusional reencounters with state B (just as with $D_{\text{rad}} \ll D_0$) so that the DEM would be valid⁹.

As a crude model, we consider diffusion with unperturbed diffusivity (D_0) over a single narrow potential barrier of width ϵ and height w , located at $r = a + \delta$. Provided that $\epsilon \ll \delta \ll a$, this model leads to⁹

$$\tau_{\text{res}} = \frac{\epsilon \delta}{D_0} \exp\left(\frac{w}{kT}\right) \quad (23)$$

In order to yield $\tau_{\text{res}} \approx 50$ ns, as deduced from the experimental data, eq. (23) requires, for $\delta < 1$ nm and $\epsilon/\delta < 0.1$, that $w \gtrsim 20$ kJ mol⁻¹. If this barrier were entirely due to packing effects, as in a hard-sphere fluid in contact with a hard wall²³, then w/kT would be independent of temperature. The activation energy for $R_s(0)$, which for large particles is associated with τ_{res} , would then be determined by the preexponential factor in eq. (23), in accordance with the experimental observations. However, the required barrier height corresponds to a local density ratio between primary maximum and minimum of order 10^3 , which does not seem consistent with a pure packing effect. (Simulation

studies²⁵⁻²⁷ of model water in contact with structureless walls yield 5 or less for this ratio.) It is conceivable that specific surface interactions could enhance the barrier, but then w (rather than w/kT) would be essentially temperature independent and would thus contribute to the activation energy for τ_{res} , in contrast to what is observed. In conclusion, then, this crude analysis indicates that dynamic correlations (leading to $D_{rad} \ll D_0$) are more important than static correlations (leading to an effective potential barrier) in determining the dynamics of radial water displacements near a surface.

3.8. PROTEIN SOLUTIONS

Because of their spherical shape and relatively uniform surface structure, colloidal silica particles constitute ideal model systems. One may ask, however, whether the picture of water dynamics emerging from studies of this model system applies also to less idealized systems such as globular proteins.

Water¹⁷O relaxation data have been reported from solutions of several medium-sized proteins^{5,8} as well as the large protein hemocyanin⁸. For the medium-sized proteins (1.5-3 nm radii) one obtains⁵ $R_s(0)/\phi$ values in the range 2000-4000 s⁻¹, i.e. somewhat higher than for the silica particles (see Fig. 2). This difference may reflect higher values of A and δ for the proteins. The observed correlation times are close to τ_{rot} so that $R_s(0)/\phi$ should increase with a , other things being equal. Unfortunately they are not⁵! The fact that $\tau_{Bs} \approx \tau_{rot}$ implies that $\tau_{rot} < \tau_{res}$,

or, with $\delta=1$ nm, that $D_{\text{rad}}/D_0 < 0.01$. The strongly reduced radial diffusivity thus appears to be a general phenomenon. For the much larger hemocyanin molecule (effective radius ≈ 18 nm) one finds⁸ $R_S(0)/\phi \approx 7000 \text{ s}^{-1}$; about twice as much as for the smaller proteins. This difference may be related to the hollow shape of the hemocyanin molecule which allows "internal hydration". The observed correlation time is ca 50 ns, more than two orders of magnitude shorter than τ_{rot} ⁸. Hence $\tau_{\text{res}} \approx 50$ ns, which happens to be very close to what was found for the silica particles.

Our dynamic model assumes that all water molecules in the first few molecular layers constituting state B have the same low radial diffusivity. It is conceivable, however, that the observed slow dynamics is due to a relatively small number of water molecules trapped in pores or clefts at the surface or interacting in specific ways, e.g. with several charged residues. If this were the case, the fast and slow contributions to the relaxation would report on different classes of water molecules, i.e. one would have $P_{\text{Bf}} \neq P_{\text{Bs}}$. Furthermore, since the surface structure varies considerably from one protein to another $P_{\text{Bs}} A^2$ ought to vary greatly. As seen from the data in Table 1, this is not the case. In fact, the ratio of the fast and slow contributions (the latter normalized by τ_{Bs} to eliminate the dependence on protein size) is remarkably constant for all investigated proteins, indicating that both contributions report on the "normal" hydration water. The same conclusion holds for the silica particles, where R_1 and R_2 vary in a similar way with pH ⁷, despite the fact that only R_2 reports on slow motions. The observation⁷ that ^2H and ^{17}O yield the same τ_{Bs} , even though the ^2H relaxation is due mainly to silanol deuterons exchanging with adjacent water molecules, lends further support to the conclusion that a large number of water molecules are involved in the slow processes.

4. CONCLUSIONS

On the basis of a general theory of spin relaxation in locally ordered fluids, we have interpreted an extensive body of water ^{17}O relaxation data from aqueous solutions of colloidal silica particles. We have focussed on the contribution to the relaxation from slow molecular processes; modelling the translational motion of water molecules by a diffusion equation incorporating an anisotropic and position-dependent diffusivity tensor. This model is consistent with all the experimental observations and leads to the following conclusions.

- (1) The slow-motion contribution to the relaxation derives from the "normal" hydration water (i.e. the first few water layers) rather than from a small number of water molecules involved in specific interactions with the surface.
- (2) The translational mobility of the hydration water is strongly reduced compared to bulk water; the radial diffusivity by at least two orders of magnitude and the lateral diffusivity by at least one order of magnitude.
- (3) The dynamic coupling of water molecules with the rotational diffusion of the colloidal particles does not affect the relaxation for particle radii in excess of ca. 10 nm.

- (4) A dynamic model which explains the long residence time of hydration water in terms of an effective potential barrier, rather than in terms of a reduced diffusivity, is not consistent with the observed activation energy for the relaxation rate. This implies that dynamic, rather than static, many-body correlations are most important in reducing the translational mobility of hydration water.
- (5) The available water ^{17}O relaxation data from protein solutions conform to the dynamic model used to interpret the data from colloidal silica dispersions.
- (6) Because of the strongly reduced radial diffusivity near the colloid surface, it is possible to define a local relaxation rate for the hydration water and hence to use the discrete-exchange formalism.

At present there exists no successful molecular theory for the self-diffusion coefficient in a simple liquid, let alone in such an intricate liquid as water. It is therefore not surprising that the theoretical understanding of surface effects on water diffusivity is wanting. However, it is suggestive that a spherical particle immersed in a viscous fluid in contact with a wall, through the so-called hydrodynamic interaction, acquires a reduced diffusivity with the perpendicular motion being affected more than the lateral motion²¹. A qualitatively similar behaviour was seen in a computer simulation study of a simple model fluid in contact with a hard wall²⁴.

Whereas the experimental information about water diffusion perpendicular to interfaces is meager, there have been several studies of lateral water diffusion in clays²⁸, protein solutions²⁹ and lyotropic liquid crystals³⁰. Despite some variation, the overall picture is consistent with the order of magnitude reduction of D_{lat} found here.

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Table 1. Water dynamics in protein solutions from ¹⁷O relaxation.

Comparison of contributions from fast and slow processes.

Data are normalized with respect to protein concentration in mass percent (W_p)

Protein	pH	$\frac{P_{Bs}(AX)^2}{W_{PR} \cdot 10^9}$	$\frac{P_{Bf}X^2f(\tau_{Bf})^a}{W_{PR}}$	$\frac{P_{Bf}f(\tau_{Bf}) \cdot 10^9a}{P_{Bs}A^2}$
		(s ⁻² mass% ⁻¹)	(s ⁻¹ mass% ⁻¹)	(s)
parvalbumin ^b	8.99	1.9	9.0	4.8
cytochrome c ^b	5.22	1.6	8.2	5.1
"	10.49	1.9	7.3	3.9
lysozyme ^b	5.11	1.0	5.3	5.6
hemoglobin, oxy ^b	9.17	1.1	7.1	6.2
plasma albumin ^b	5.09	1.8	7.3	4.1
"	2.18	1.3	2.5	1.9
"	5.24	2.0	7.0	3.5
"	5.11	2.3	9.0	3.9
hemocyanin 1/1 ^c	9.00	5.7-2.4	10.9	1.9-4.6
hemocyanin 1/10 ^c	9.00	3.8-1.6	7.9	2.1-5.0

^a $f(\tau_{Bf}) = (1 + \frac{\eta^2}{3} - A^2)\tau_{Bf} - (1 - \frac{\eta^2}{3})\tau_F$ (see refs. 5 and 8).

^b 27°C; data from table I of ref. 5.

^c 25°C; calculated from data at 34.6 MHz in table 2 of ref. 8 using $35 \leq \tau_{Bs}/ns \leq 85$ and $R_F = 151\text{ s}^{-1}$.

FIGURE CAPTIONS

Fig. 1. ^{17}O relaxation rate, $R_s(0)$, versus colloid-to-water volume ratio, ϕ , in solutions of silica particles of radius 12.1 nm (a) and 41.2 nm (b) at 23°C . The experimental points were obtained from experimental R_2 data and fitted R_1 data of Fig. 2 in Part 1, using eq. (19). The estimated uncertainty is $\pm 5 \text{ s}^{-1}$. The curves were calculated from eq. (12) with $A\chi = 3.34 \times 10^5 \text{ Hz}$, $D_0 = 2.20 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ and other parameters as given in the inserts.

Fig. 2. Concentration-normalized ^{17}O relaxation rate, $R_s(0)/\phi$, versus silica particle radius, a , at 4°C (open symbols) and 27°C (filled symbols). Curve 1(2) was calculated from eq. (12) with $A\chi = 3.34 \times 10^5 \text{ Hz}$, $\delta = 1 \text{ nm}$, $D_{\text{rad}}/D_0 = 0.0030(0.0045)$ and $D_{\text{lat}}/D_0 = 0.10(0.065)$. The upper boundary of the shaded areas corresponds to $D_{\text{rot}} = 0$. Curve 3 was calculated from eq. (18) with $\delta(A\chi)^2 = 111 \text{ ms}^{-2}$, $\delta^2/D_{\text{rad}} = 1.53 \times 10^{-7} \text{ s}$ and $D_{\text{lat}} = 2.90 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$. Curve 4 was calculated from eq. (18) with $D_{\text{rot}} = 0$ and $\delta^3(A\chi)^2/D_{\text{rad}} = 1.70 \times 10^{-5} \text{ ms}^{-1}$ and $\delta^2 D_{\text{lat}}/D_{\text{rad}} = 4.44 \times 10^{-17} \text{ m}^2$.

Fig. 3. Frequency dependence of the ^{17}O relaxation rate R_{1s} in a solution of silica particles of radius 12.2 nm at $\phi = 0.113$ and 25°C . The data were obtained from Table 2 in Part 1. The solid curve is the DEM dispersion, eq. (20), with an effective correlation time $\tau_{Bs} = 32.5 \text{ ns}$, calculated with the fitted parameters from curve 1 of Fig. 2. The dotted curve is the dispersion predicted theoretically with $D_{\text{rad}} = D_{\text{lat}} = D_0$ and $\delta = 3.8 \text{ nm}$ (cf. Fig. 1a).

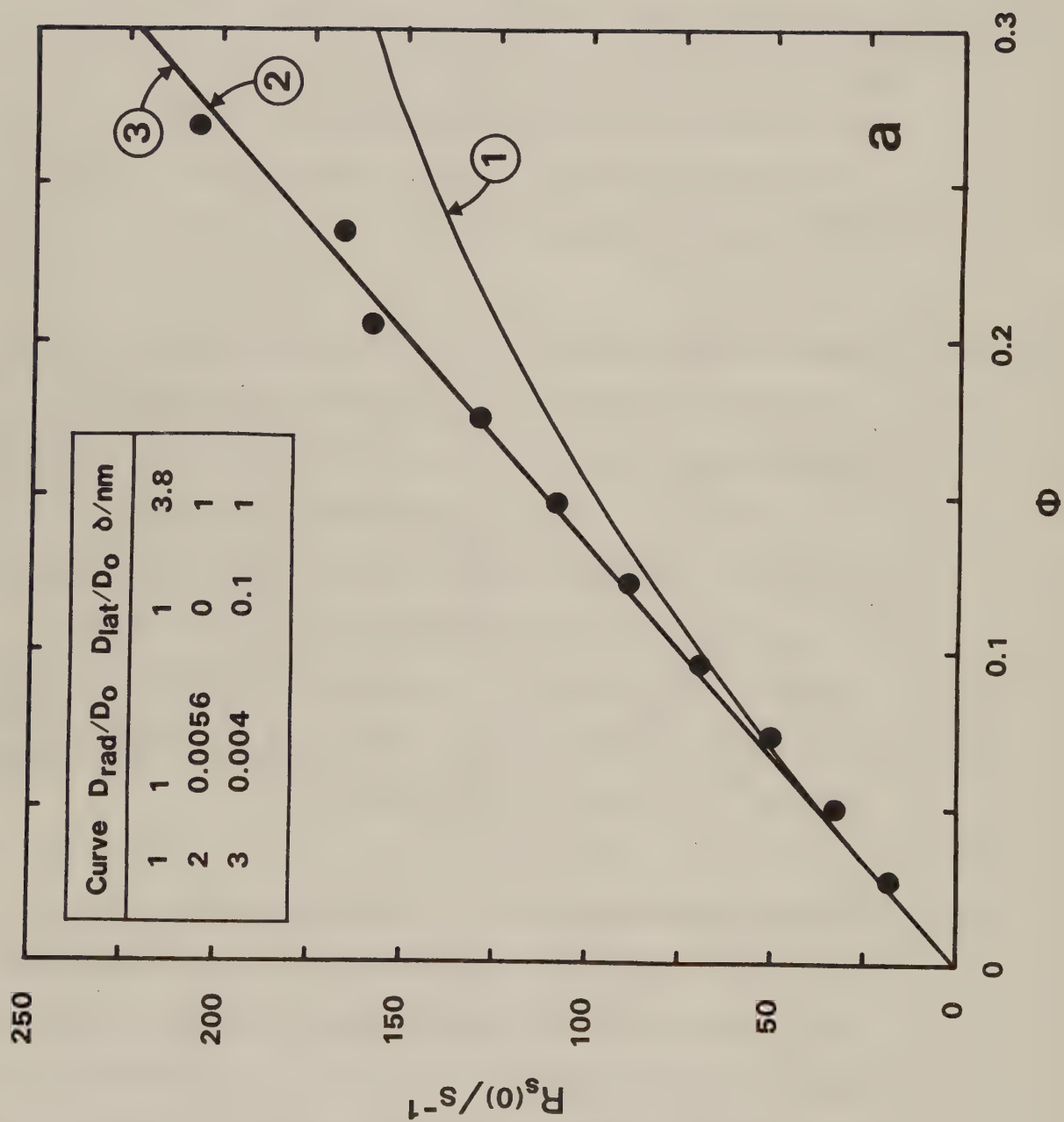


FIGURE 1a

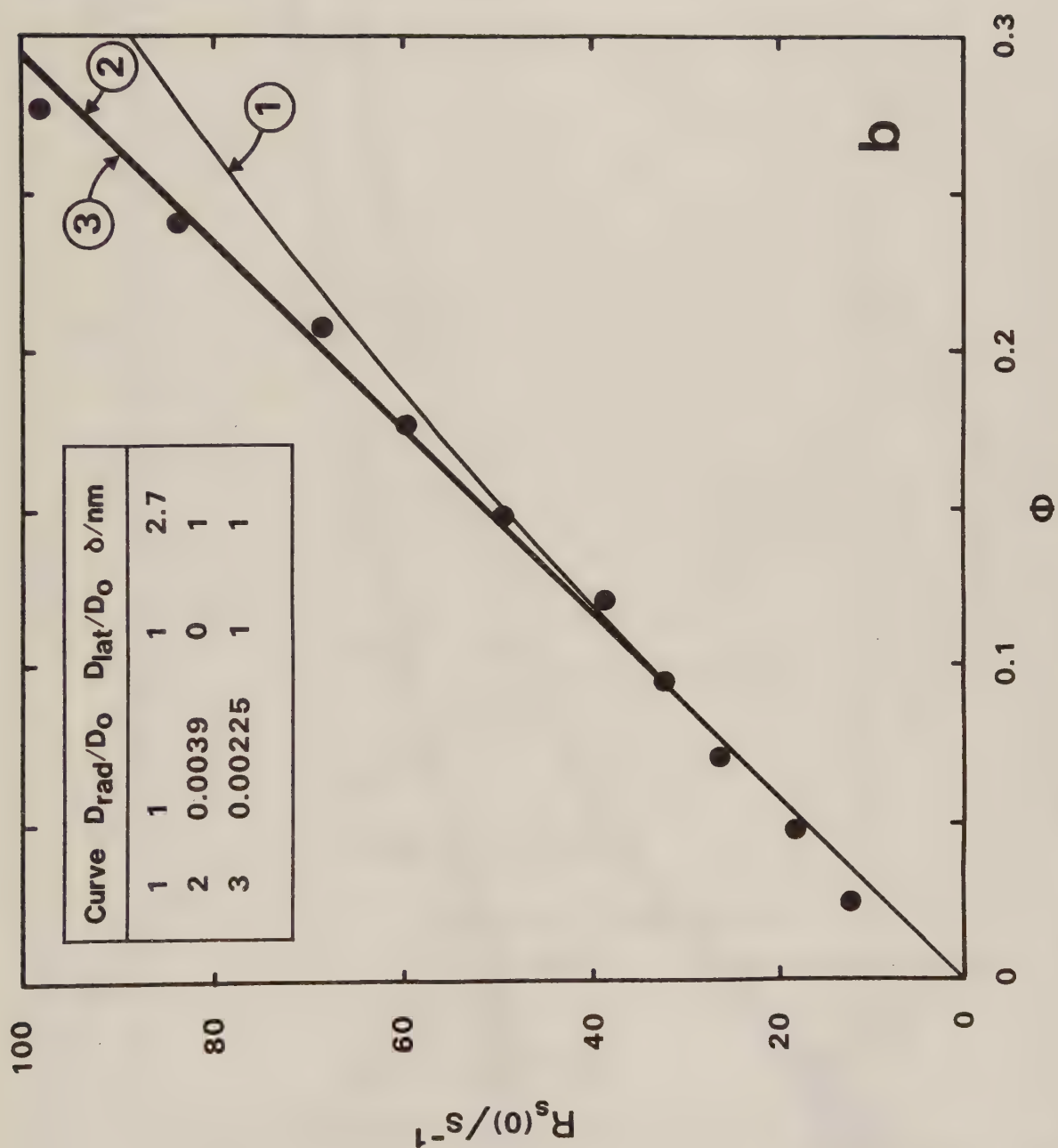


FIGURE 1b

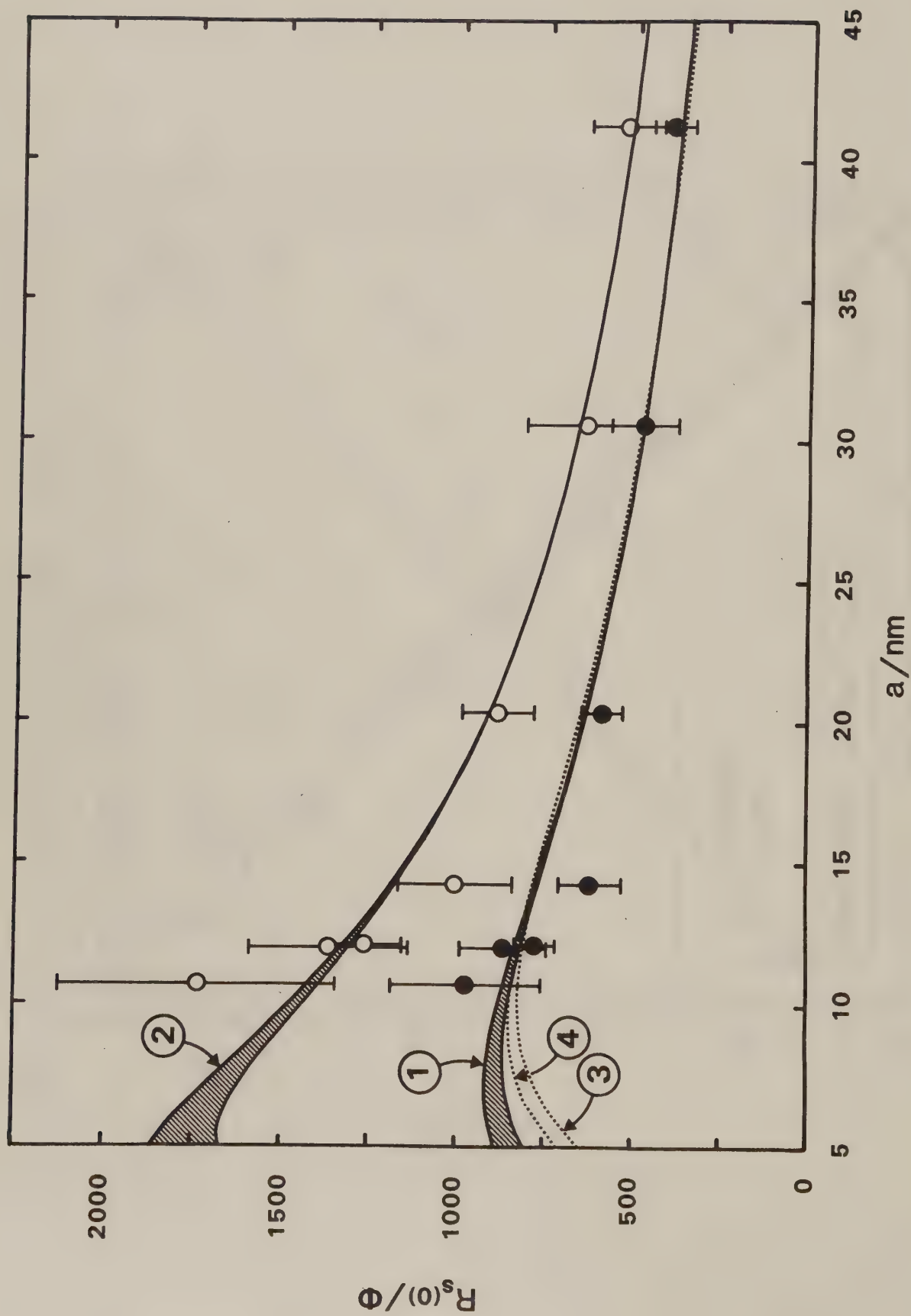


FIGURE 2

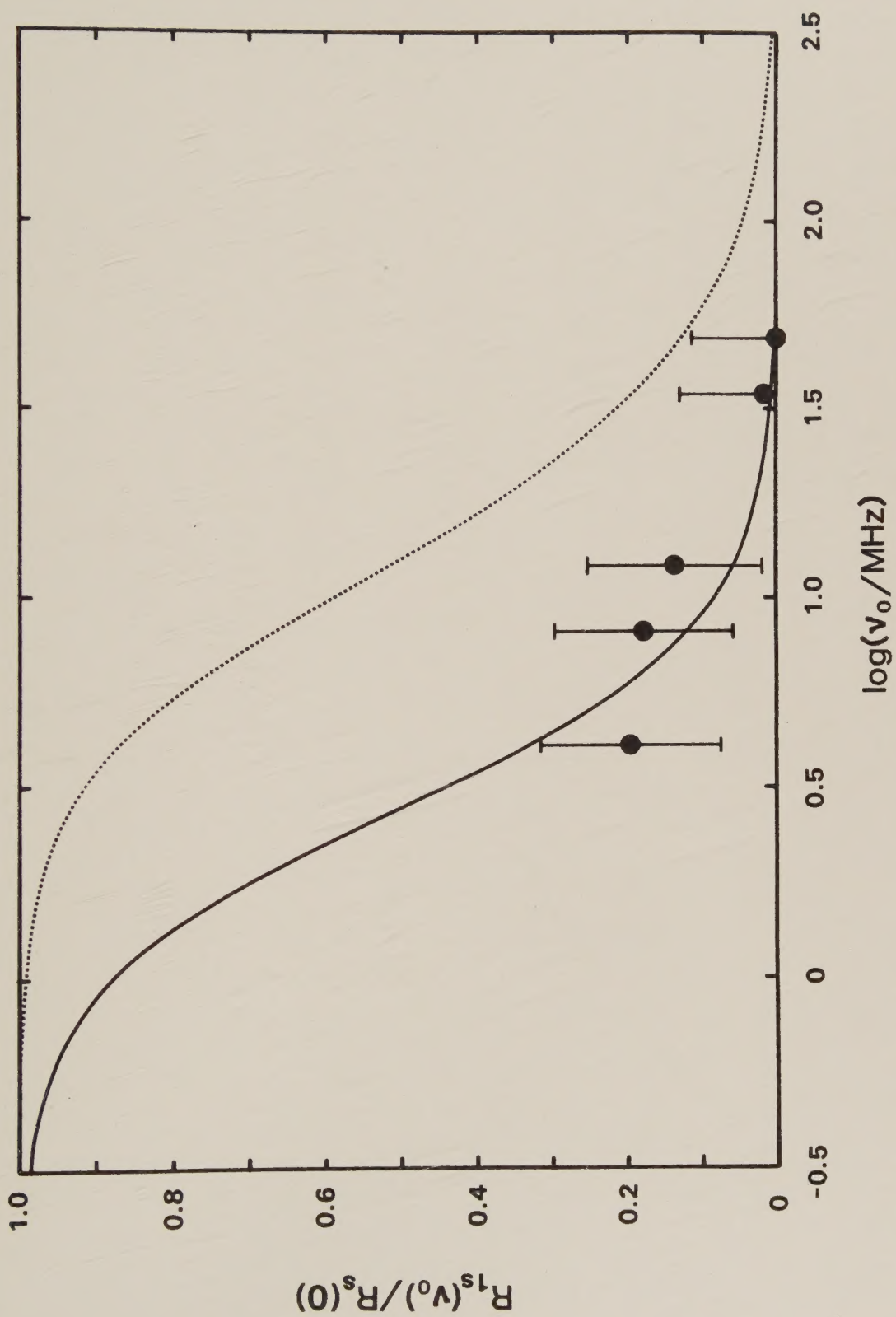


FIGURE 3

